Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note **REG2004-06 revision**

Clothianidin
Poncho 600 Seed Treatment Insecticide

The insecticide active ingredient, clothianidin, and the associated end-use product (EP), Poncho 600 Seed Treatment Insecticide, for seed treatment to control flea beetle on canola/rapeseed and to control corn rootworm, corn flea beetle, black cutworm, seed corn maggot, wireworm and white grub on corn have been granted temporary registration under Section 17 of the Pest Control Products (PCP) Regulations.

This Regulatory Note provides a summary of data reviewed and the rationale for the regulatory decisions for these products.

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Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued temporary registrations for the technical grade insecticide clothianidin, manufactured by Sumitomo Chemical Takeda Agro Company, Ltd. (Sumitake), and the associated end-use product, Poncho 600 Seed Treatment Insecticide, manufactured by Bayer CropScience, for the control of flea beetle on canola/rapeseed and for the control of corn rootworm, corn flea beetle, black cutworm, seed corn maggot, wireworm and white grub on corn.

These products were reviewed jointly by the PMRA and the United States Environmental Protection Agency (USEPA) within the North American Free Trade Agreement Technical Working Group on Pesticides (NAFTA TWG) Joint Review Program, as a Group 2 Joint Review of Non-Reduced Risk Chemicals.

Bayer CropScience formatted and submitted the applications for review in the Organisation for Economic Co-operation and Development (OECD) dossier format (Revision 1), a universal format for pesticide dossiers developed in conjunction with all OECD-member countries. These dossiers were partially provided in electronic format. While the PMRA accepts dossiers in the PMRA format or the OECD format, applicants are encouraged to use the OECD dossier format. As well, Canada and the United States shared reviews of this product with Australia.

Methods for analyzing clothianidin in environmental media are available to research and monitoring agencies upon request to the PMRA.

Bayer CropScience will be carrying out additional studies as a condition of this temporary registration. Following the review of this information, the PMRA will publish a proposed regulatory decision document (PRDD) and request comments from interested parties before proceeding with a final regulatory decision.

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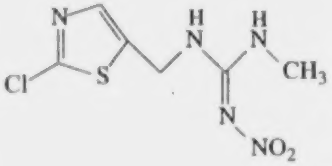
1.0 The active substances, its properties and uses

1.1 Identity (OECD 2.1.1)

Technical grade active ingredient (TGAI) identification

Active substance	Clothianidin
Function	Insecticide
Chemical name	
1. International Union of Pure and Applied Chemistry (IUPAC)	(E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
2. Chemical Abstracts Service (CAS)	(E)-N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl-N"-nitroguanidine
CAS number	210880-92-5
Molecular formula	$C_6H_8ClN_5O_2S$
Molecular weight	249.68
Structural formula	
Nominal purity of active	Clothianidin 97.5% (Limits 95–100%)
Identity of relevant impurities of toxicological, environmental or other significance	The technical grade clothianidin does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances

1.2 Physical and chemical properties (OECD 2.1.2)

Technical product: Clothianidin (TI-435)

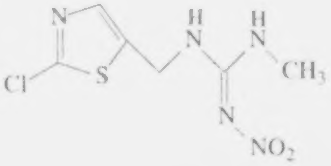
Property	Result	Comment
Colour and physical state	Clear and colourless solid	
Odour	Odourless	

Property	Result	Comment
Melting point or range	176.8°C	
Boiling point or range	N/A	
Density	1.59 g/mL	
Vapour pressure	1.3×10^{-10} Pa at 25°C	Not volatile
Henry's Law constant at 20°C	9.8×10^{-16} atm m^3/mole	Non-volatile from water and moist soil surfaces
Ultraviolet–visible spectrum	pH λ_{max} (nm) acidic 265.5 basic 246.0 Not expected to absorb at λ higher than 300 nm	
Solubility in water at 20°C	0.327 g/L	Very soluble in water
Solubility in organic solvents at 25°C	Solvent mg/L heptane < 0.00104 xylene 0.0128 dichloromethane 1.32 methanol 6.26 octanol 0.938 acetone 15.2 ethyl acetate 2.03	
n-Octanol–water partition coefficient (K_{ow}) at 25°C	$K_{ow} = 5$	Low potential to bioaccumulate
Dissociation constant (pK_a)	$pK_a = 11.09$ at 20°C	Not likely to dissociate in acidic and neutral solutions
Stability (temperature, metal)	Stable to zinc, zinc ions, iron, iron ions, aluminum and aluminum ions at 25°C and 54°C for 14 days	

1.0 The active substances, its properties and uses

1.1 Identity (OECD 2.1.1)

Technical grade active ingredient (TGAI) identification

Active substance	Clothianidin
Function	Insecticide
Chemical name	
1. International Union of Pure and Applied Chemistry (IUPAC)	(E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
2. Chemical Abstracts Service (CAS)	(E)-N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl-N"-nitroguanidine
CAS number	210880-92-5
Molecular formula	$C_6H_8ClN_5O_2S$
Molecular weight	249.68
Structural formula	
Nominal purity of active	Clothianidin 97.5% (Limits 95–100%)
Identity of relevant impurities of toxicological, environmental or other significance	The technical grade clothianidin does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances

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Boiling point or range	N/A	
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Vapour pressure	1.3×10^{-10} Pa at 25°C	Not volatile
Henry's Law constant at 20°C	9.8×10^{-16} atm m^3/mole	Non-volatile from water and moist soil surfaces
Ultraviolet–visible spectrum	pH λ_{max} (nm) acidic 265.5 basic 246.0 Not expected to absorb at λ higher than 300 nm	
Solubility in water at 20°C	0.327 g/L	Very soluble in water
Solubility in organic solvents at 25°C	Solvent mg/L heptane < 0.00104 xylene 0.0128 dichloromethane 1.32 methanol 6.26 octanol 0.938 acetone 15.2 ethyl acetate 2.03	
n-Octanol–water partition coefficient (K_{ow}) at 25°C	$K_{ow} = 5$	Low potential to bioaccumulate
Dissociation constant (pK_a)	$pK_a = 11.09$ at 20°C	Not likely to dissociate in acidic and neutral solutions
Stability (temperature, metal)	Stable to zinc, zinc ions, iron, iron ions, aluminum and aluminum ions at 25°C and 54°C for 14 days	

End-use product: Poncho 600 Seed Treatment Insecticide

Property	Result
Colour	Off-white
Odour	Latex paint like
Physical state	Viscous liquid
Formulation type	Liquid suspension
Nominal guarantee	Clothianidin 600 g/L (Limits 580–630 g/L)
Formulants	The product does not contain any USEPA List 1 or 2 formulants or formulants known to be TSMP Track 1 substances.
Container material and description	High-density polyethylene (HDPE)—100 L drum, 10 L and 3.79 L jugs
Specific gravity	1.2632 at 20°C
pH of 10% dispersion in water	5.5
Oxidizing or reducing action	The TGAI and all formulants are not oxidizers or reducers.
Storage stability	A one-year storage stability study is pending.
Explodability	Not explosive

1.3 Details of proposed uses and further information

Poncho 600 Seed Treatment Insecticide (commercial class) is a flowable suspension formulation of clothianidin. This product is used for seed treatment to control several insect pests on canola/rapeseed and corn. The proposed use-site category for this product is USC 10—Seed Treatments Food and Feed.

See Section 7.1.1 for detailed information on the proposed uses and application rates of this product.

2.0 Methods of analysis

2.1 Analytical methods for analysis of the active substance as manufactured (OECD 2.2.1)

Four high-performance liquid chromatography (HPLC) methods with ultraviolet (UV) detection were used for the determination of the active ingredient and process-related

End-use product: Poncho 600 Seed Treatment Insecticide

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1.3 Details of proposed uses and further information

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See Section 7.1.1 for detailed information on the proposed uses and application rates of this product.

2.0 Methods of analysis

2.1 Analytical methods for analysis of the active substance as manufactured (OECD 2.2.1)

Four high-performance liquid chromatography (HPLC) methods with ultraviolet (UV) detection were used for the determination of the active ingredient and process-related impurities present in the product at levels above 0.1%. The methods have been assessed to be fully validated. The company has also provided an inductively coupled plasma (ICP) method for the determination of metal impurities and an HPLC method for the determination of inorganic ions. A fully validated gas chromatography (GC) method with thermal energy analyzer (TEA) detection was used for the analysis of non-polar and polar N-nitrosamines.

2.2 Analytical methods for formulation analysis (OECD 2.2.2)

An HPLC method with UV detection was used for the determination of the active. The method was assessed to be specific, precise and accurate for use as an enforcement analytical method.

2.3 Analytical methods for residue analysis (OECD 2.2.3)

2.3.1 Analytical methods for residues in environmental samples

A high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was used for the determination of clothianidin (TI-435) and its transformation products, TZNG, TZMU, MNG and TMG, in soil samples. TZNG, TZMU, MNG and TMG were found at concentrations > 10% in the environmental fate studies conducted with TI-435. Full validation data and all necessary chromatograms were provided. The method was assessed for validation and found to be acceptable. The method was also validated by an independent laboratory and found to be acceptable for the determination of clothianidin and its metabolites in soil samples.

Two HPLC methods with UV detection were used for the determination of the active ingredient TI-435 in surface water and for TZNG in test water from aquatic study. No accuracy data were provided for the determination of TZNG. However, since there are no clean-up procedures in a sample preparation, this requirement was waived. All other validation data for both methods were provided. The methods were assessed for validation and found to be acceptable.

Two HPLC analytical methods were used for the determination of the active ingredient TI-435 in plant matrices. Full validation data and all necessary chromatograms were provided. The methods were assessed for validation and found to be acceptable. The methods were successfully validated by an independent laboratory.

Two HPLC methods were used for animal matrices. The first HPLC method with UV detection was used for the determination of the active ingredient TI-435. The second HPLC method with MS detection was used for the determination of the active ingredient and for three of its metabolites (TZG, TZU and PTMG). The methods were assessed for validation and found to be acceptable.

2.3.2 Multiresidue methods for residue analysis

TI-435 and its metabolites MNG, TZG, TZNG, TZU, and ATMG-Pyr were analyzed according to the United States Food and Drug Administration (USFDA) *Pesticide Analytical Manual* (PAM), Volume 1, Multiresidue Methods (1994). Under Protocol A, TI-435, MNG, TZG, TZNG, TZU and ATMG-Pyr were determined not to be naturally fluorescent. Protocol B was not tested because TI-435 and its metabolites are not acid or phenol compounds. TI-435, MNG, TZG, TZNG, TZU and ATMG-Pyr were tested through Protocol C; all compounds except MNG had acceptable retention times on at least one column. Therefore, TI-435, TZG, TZNG, TZU and ATMG-Pyr were tested through Protocols D and E. None of the compounds were adequately recovered using Protocol D. Based on poor recoveries using Protocol E (Florisil clean-up), no further work was conducted using Protocols E and F. Protocol G was initiated with TZU because it is the only compound that is a substituted urea; however, the TZU peak response was not significant enough to warrant analysis through the remainder of Protocol G. Therefore, TI-435 and the metabolites TZG, MNG, TZNG, TZU and ATMG-Pyr were not adequately recovered using any of the multiresidue methods.

2.3.3 Methods for residue analysis of plants and plant products

Method 00552, used for data gathering and proposed as an enforcement method, determines residues of TI-435 in plant commodities. Briefly, residues are extracted with acetonitrile/water, they are filtered, and the filtrate is concentrated for clean-up through a ChemElut column eluted with cyclohexane/ethyl acetate. Residues are analyzed by HPLC using a C18 column, a gradient mobile phase of acidic water and acetonitrile, with identification and quantitation by MS/MS using external bracketing standards of TI-435. The validated limit of quantitation (LOQ) without using an internal standard is 0.02 ppm for corn plant, cob, grain and straw; rape forage, straw and seed; sugar beet tops and root; sunflower plant and seed; as well as wheat forage, grain and straw. A modification of the method has been submitted (M001) that adds the use of an internal standard, d_3-TI-435. With the use of the internal standard, the validated LOQ is 0.01 ppm, with the exception of 0.02 ppm for wheat straw. Acceptable method validation has been submitted for Method 00552 (and modification M001). Validation data were included for the determination of the parent compound, using external bracketing standards and with an internal standard. A successful independent laboratory validation (ILV) has been completed with corn grain along with acceptable radiovalidation data for apple, corn forage, stover and grain. No interferences were observed in any of the studies, including an interference study that investigated 133 compounds.

2.3.4 Methods for residue analysis of food of animal origin

Method 00624, proposed as an enforcement method, determines residues of TI-435 and its metabolites, TZG [(2-chloro-5-thiazolyl)methyl guanidine], TZU [2-chloro-5-thiazolyl)methyl urea] and ATMG-pyruvate [*N*'-[(2-chlorothiazol-5-ylmethylamino) (methylamino)methylene]-2-oxopropano hydrazide], in ruminant commodities. Briefly, residues in tissues are extracted with acetonitrile/water, and the extract is concentrated for clean-up through a stryene divinyl benzene polymer solid phase extraction column for environmental testing (Bond Elut™ ENV) column eluted with acidic water/methanol. Fat samples are extracted with acetonitrile/water and hexane, and the extract is partitioned between acetonitrile and hexane; the acetonitrile/water phase is concentrated for clean-up through an ENV column. Milk samples are simply diluted with water and applied to an ENV column for clean-up. Residues are analyzed by HPLC using a C18 column, a gradient mobile phase of acidic water and acetonitrile, followed by identification and quantitation using MS/MS with an external calibration curve of standards and the use of an internal standard. The reported method limit of detection (LOD) for TI-435, TZG, TZU, and ATMG-pyruvate was 0.002 ppm each for milk and 0.005 ppm each for meat, liver, kidney and fat, and the validated LOQ was 0.01 ppm for each analyte in milk and 0.02 ppm for each analyte in animal tissues. Adequate method validation has been submitted for Method 00624. Validation data were included for the metabolites, TZG, TZU and ATMG-pyruvate, as well as for the parent compound. Overall recoveries (all analytes) at the method LOQ and 10 times the LOQ ranged from 86 to 110% (milk), from 78 to 109% (muscle), from 76 to 121% (liver), from 81 to 108% (kidney) and from 73 to 105% (fat); the coefficient of variance ranged from 3.2 to 10.9%. A successful ILV has been completed with liver, and no interferences were observed in a study that investigated potential interferences from 163 compounds.

3.0 Impact on human and animal health

3.1 Integrated toxicological summary

A detailed review of the toxicological database for the new insecticide, clothianidin (TI-435), was conducted. The database is complete, consisting of the full array of toxicity studies currently required for regulatory purposes. The studies were carried out in accordance with currently accepted international testing protocols and good laboratory practices. The scientific quality of the data is high and the database is considered adequate to define the majority of the toxic effects that may result from exposure to this chemical. However, clarification of certain findings and additional studies may be required as outlined below.

In the rat, absorption and excretion of single low or high, or repeated oral doses of TI-435 was very rapid. Whole body radio-autography and time-course plasma studies revealed rapid absorption and distribution of administered radioactivity to all organs and tissues, followed by rapid excretion with reduction to background levels in most tissues and organs within 24 hours. The metabolites identified were consistent with Phase I

processes. In mice, TI-435 was readily absorbed and excreted within 168 hours of administration a single low oral dose. Urine was the major route of excretion of TI-435, accounting for most of the administered radioactivity, with the balance of recovery in the feces. Neither TI-435 nor its metabolites appeared to exhibit potential for bioaccumulation. The major metabolites found in both the urine and feces of rats and mice were the parent compound (TI-435) and
TZNG [N-(2-chlorothiazol-5-methyl)-N′-nitroguanidine].

TI-435 was of high acute toxicity by the oral route, of low acute toxicity by dermal and inhalation exposure, minimally irritating to the eyes and to the skin, and not considered to be a skin sensitizer when tested up to a 20% concentration. The mouse was more sensitive to the toxic effects of clothianidin after acute oral exposure with an LD_{50} in the order of a magnitude lower than that observed in the rat. Clinical signs of acute toxicity appeared to be largely related to effects on the central nervous system. The formulated product, Poncho 600 containing 48% TGAI, was considered to be of moderate acute toxicity by the oral route, of low acute toxicity by dermal and inhalation exposure, non-irritating to the skin and eyes, and not a skin sensitizer.

A short-term dermal toxicity study in rats showed no skin irritation in any of the test groups after repeated applications of TI-435 to the shaved skin of albino rats. Males had reduced body-weight gains at the limit dose of 1000 mg/kg bw/d.

Short-term dietary studies indicated that the hematopoietic system was the target of toxicity in dogs, mice and rats. Rats exhibited thymus effects in the form of decreased organ weight and cellularity. Liver effects included enzyme induction and altered clinical chemistry values. Anemia and decreased white blood cell parameters were salient effects noted in dogs, with associated congestion and hemorrhage of the bone marrow. Decreased body weights, food consumption and body-weight gains were noted in all species tested.

Toxic effects were observed in several organs with little consistency among studies or between species. The liver, kidney, reproductive organs, gastrointestinal tract and immune system showed evidence of being targets of the toxic effects of clothianidin.

Batch # 30034708 tested positive in both a bacteria assay and in vitro mammalian cell cultures. This batch of chemical was negative in assays done in vivo (mouse micronucleus test and unscheduled DNA synthesis assay). Several other batches of TI-435 tested negative in similar assays. Ames tests performed on eight intermediates or metabolites of TI-435 were all negative as well. However, there is uncertainty regarding the genotoxic potential of batch # 30037120, which was used for chronic toxicity testing, as only one bacteria mutation assay was available for this batch of chemical. This batch of chemical contains potential impurities of toxicological concern. Further testing on this batch of chemical will be required prior to rendering a final decision as to the genotoxic potential of TI-435.

Short-term feeding studies were available for both batch # 30034708 (used for most of the genotoxicity package) and batch # 30037120 (used in chronic feeding studies). Studies using batch # 30034708 were conducted at a different facility than the studies using batch # 30037120, and the toxicity was notably different between these studies. The difference in the toxicity between batches adds uncertainty to the chemical identification and consistency in the toxicological database.

The neurotoxic potential of TI-435 was investigated in acute (mice and rats), subchronic and developmental neurotoxicity studies. Neurotoxicity in an acute oral study in rats was demonstrated as decreased arousal, tremors, decreased activity, and ataxia. In a subchronic neurotoxicity study, no treatment-related signs of neurotoxicity were observed. In a developmental neurotoxicity study, reduced motor activity and acoustic startle response in females was noted at maternally non-toxic doses. In an acute neurotoxicity study in mice, decreased spontaneous motor activity, tremors and deep respirations were observed rapidly following dosing. There were no neuropathological findings in any of these studies.

Fetotoxicity (decreased body-weight gains, delayed sexual maturation [males], decreased absolute thymus weights in F_1 pups of both sexes and an increase in stillbirths in both generations) occurred at maternally non-toxic doses in a two-generation reproductive toxicity study. The parents demonstrated decreased absolute and relative thymus weights in both sexes at the highest dose tested. The rat developmental toxicity study demonstrated effects on maternal body-weight gain and decreased food consumption, but there was no evidence of fetotoxicity or teratogenicity at the highest dose tested. The rabbit developmental toxicity study had greater evidence of toxicity than the rat study with mortality, premature deliveries, decreased gravid uterine weights, increased incidences of clinical signs and decreased food consumption in dams. There was also an increased litter incidence of a missing lobe of the lung and decreased ossified sternal centra per fetus. TI-435 was not teratogenic in the rat and rabbit developmental toxicity studies.

Effects consistent with endocrine disruption were also noted in rodents and dogs. Testicular atrophy and reduced colloid in the seminal vesicles and prostate occurred in male rats and mice in the short-term studies. Ovaries were small, lower in weight and had no corpora lutea, uterus walls were thinner and reduced mural thickness was observed in the short-term rat study. These studies were the only studies in the database conducted using batch # 30033623. Testes and ovary weights were lower in dogs in subchronic testing using batch # 30037120. Subchronic studies were conducted in mice and rats using batch # 30034708. The mouse study demonstrated ovaries with lower numbers of corpora lutea, lower numbers of large follicles and more signs of follicular degeneration. In the rat study, uterus and ovary weights were higher while most females in treated groups were in the proestrus phase of the reproductive cycle compared with most females in control group being in the diestrus phase. Fluid distension of the uterus and higher numbers of corpora lutea were observed in rats in the subchronic study. In chronic testing in rats, interstitial cell hyperplasia in the ovary was increased in incidence in all treated groups of females, while chronic testing in mice demonstrated cervical fibromuscular hyperplasia. In a two-generation reproductive toxicity study, preputial separation and vaginal opening were delayed in pups, and sperm motility was decreased in both generations. The delay in sexual maturation was noted in the developmental neurotoxicity study as well; however, the control group animals developed at a rate in excess of the range of historical control values, making the trend in this study of limited use. Other signs of possible endocrine disruption included increased adrenal weights with noted congestion, and increased thyroid weights with noted cysts and adenomas. These indications of endocrine disruption were consistent with findings in the database of a similar chloro-nicotinic compound.

There was no evidence of oncogenicity after chronic administration of TI-435 in mice. Systemic toxicity was manifested as reduced body weights, body-weight gains, vocalizations and cervical fibromuscular hyperplasia. In rats, females showed decreased body weights and food consumption, liver toxicity and ovarian interstitial gland hyperplasia, while males demonstrated decreased body weights and food consumption, liver toxicity, stomach edema and hemorrhage as well as kidney mineralization and hyperplasia. Long-term studies in rats provided quantitative evidence of treatment-induced oncogenicity (thyroid C-cell adenomas in females). The tumour incidence exceeded the range of both the laboratory and animal supplier historical control values at the two highest doses tested. There was no progression of preneoplastic lesions to the adenomas, and the tumour was evident in only one gender of one species. The adverse effects noted in endocrine tissues, including the thyroid, may indicate a hormonal influence on tumour promotion. Mechanistic data was not available to investigate this hypothesis.

The batch of chemical used in the long-term studies contained several contaminants that may have tumour causing potential, based on chemical structural analysis. This batch of chemical was not tested in a genotoxicity battery but was subjected to an Ames test. It is plausible that the tumorigenic response observed was contaminant mediated. Accordingly, the PMRA has requested a genotoxicity battery on this batch and Bayer CropScience is in the process of conducting the studies. Until the contaminant issue has been resolved, a conservative cancer risk assessment is being applied to the database in the interim. Unit risks for TI-435, denoted by Q_1^* (representing the upper 95% confidence limit on the slope of the dose-response curve in the low-dose region) were calculated based on the bioassay data from the rat oncogenicity study with the high-dose animals removed due to excessive toxicity at this dose level. The unit risk of 2.33×10^{-3} $(\text{mg/kg bw/d})^{-1}$ will be applied in the risk assessments for dietary and worker exposure. Following the submission of a complete genotoxicity battery of studies conducted with batch # 30037120 and resolution of the genotoxicity/cancer issue, the cancer risk assessment approach will be reassessed. In addition, submission of mechanistic data to provide evidence for a non-genotoxic mode of tumorigenesis is an option that the companies may wish to use to address the genotoxicity concern.

There was evidence of effects on the immune system. Decreased absolute and adjusted thymus and spleen weights were observed in multiple studies. Lower white blood cell counts were a consistent finding in dogs. In the short-term dog study, findings included diffuse hypocellularity of bone marrow and depletion of lymphoid cells in the thymus, spleen and mesenteric lymph nodes. Thymic involution and lymphoid cell depletion in the thymus were observed in the short-term mouse and rat studies. In addition, juvenile rats in the two-generation reproduction study appeared to be more susceptible to these effects. Additional testing is required to assess immune system function in adults and in young animals following developmental exposures. A developmental immunotoxicity study is required to address this issue.

The company has applied for the approval of clothianidin TGAI manufactured in Germany in addition to the original Sumitake process. Batch analysis demonstrated that each process generated distinct impurity profiles in pilot-scale production. Toxicological studies are available for three batches produced with one pilot-scale manufacturing process only (Sumitake). Long-term studies were conducted with one batch of chemical only, while short-term and sub-chronic studies conducted with other batches of chemical demonstrated different end-points of toxicity. The inconsistent toxicological findings may be the result of differences in contaminants in the Sumitake TGAI. The new German source of TGAI contains fewer contaminants at lower concentrations than the original Sumitake TGAI and likely has a cleaner toxicological profile. Although the new manufacturing method has reduced the presence of impurities, the impurity profile may change during full-scale production. As a result, additional toxicity studies may be required to further characterize the technical material.

3.2 Determination of acceptable daily intake (ADI)

The acceptable daily intake of 0.0327 mg/kg bw/d is derived from the two generation reproduction study conducted in rats. The no observed adverse effect level (NOAEL) for this study was 9.8 mg/kg bw/d in males and 11.5 mg/kg bw/d in females, based on decreased body-weight gains, delayed sexual maturation (males) and decreased absolute thymus weights in F_1 pups of both sexes as well as an increase in stillbirths in both generations at doses of 31.2 mg/kg bw/d in males and 36.8 mg/kg bw/d in females. For the calculation of the ADI, a target margin of exposure (MOE) of 300 is required. The additional 3× safety factor is recommended for the protection of infants and children, based on the observed increased susceptibility of the neonates to TI-435 following repeated oral exposures in the two-generation reproduction study and for the required developmental immunotoxicity study.

The acceptable daily intake is calculated according to the following formula:

$$\text{ADI} = \frac{\text{NOAEL}}{\text{UF/SF}} = \frac{9.8\ \text{mg/kg bw/d}}{300} = 0.0327\ \text{mg/kg bw/d of TI-435.}$$

3.3 Acute reference dose (ARfD)

The acute reference dose (ARfD) for females in the 13 + age group is 0.25 mg/kg bw. The toxicology endpoint for acute dietary exposure for females in this age group is based on an increased litter incidence of a missing lobe of the lung in the rabbit developmental toxicity study. This endpoint is considered appropriate for this population subgroup because the observed developmental effects may occur following a single dose. In addition, the route of administration is oral, which is appropriate for dietary considerations. With a NOAEL of 25 mg/kg bw/d and a LOAEL of 75 mg/kg bw/d, the dose spread is not considered to be large; therefore, the endpoint is not considered to be overly conservative. Other effects observed at the same dose level were premature deliveries, decreased gravid uterine weights and decreased litter average for ossified sternal centra per fetus. These are not considered to be single dose effects.

The ARfD for the general population is 0.25 mg/kg bw. The endpoint is based on a combined neurotoxicity/pharmacology study in CD-1 mice given a single gavage dose of TI-435. The NOAEL for this study was 25 mg/kg bw based on evidence of decreased spontaneous motor activity, tremors, deep respirations decreased reactivity, decreased grooming, decreased muscle tone, prone position, staggering gait, mydriasis, and hypothermia at the LOAEL of 50 mg/kg bw. For the calculation of the ARfD, a target MOE of 100 is required.

The ARfD is calculated according to the following formula:

$$\text{ARfD} = \frac{\text{NOAEL}}{\text{UF/SF}} = \frac{25\ \text{mg/kg bw}}{100} = 0.25\ \text{mg/kg bw of TI-435.}$$

3.4 Toxicological endpoint for assessment of occupational and bystander risks – AOEL/MOE (OECD 2.3.4)

There is a potential for an intermediate duration of exposure to workers in seed treatment facilities performing various tasks for three to five months of the year. Dermal and inhalation are the predominant routes of exposure. On-farm seed treatment by farmer or custom applicators is not proposed for this product and therefore not evaluated herein.

There is also a potential for a short duration of exposure to workers planting treated seed in early spring from one to three weeks per year. The principal route of exposure is dermal.

For the non-cancer risk assessment, it was considered appropriate to use the NOAEL of 9.8 mg/kg bw/d in the occupational risk assessment for short- to intermediate-term exposure durations. This is based on the nature of the findings in the rat reproductive toxicity study and in consideration of the immunotoxicity observed throughout the database. In the rat study, pups had an increased incidence of stillbirths, delayed sexual maturation, decreased thymus weights and reduced body-weight gains at the LOAEL of

31.2/36.8 mg/kg bw/d (male/female). A developmental immunotoxicity study is required, based on the evidence of increased susceptibility of the young (decreased thymus weights at the pup LOAEL) and in consideration of similar effects observed throughout the toxicological database for TI-435. The target MOE is 300. The additional 3× safety factor is recommended for the protection of infants and children, based on the observed increased susceptibility of the neonates to TI-435 following repeated oral exposures in the two-generation reproduction study and for the required developmental immunotoxicity study.

It was considered appropriate to ensure that the occupational risk assessment also addressed workers who may have occasional elevated exposures. The relevant endpoint for these exposures is the NOAEL used in establishing the dietary ARfD (i.e., NOAEL of 25 mg/kg bw from the acute neurotoxicity study in mice and the developmental study in rabbits). The target margin of exposure for this endpoint is 100.

For the cancer risk assessment, in view of the uncertainty regarding the mode of action leading to the observed tumour response, it was considered appropriate to use a quantitative approach to the cancer risk assessment. Unit risks for TI-435, denoted by Q_1^* (representing the upper 95% confidence limit on the slope of the dose-response curve in the low-dose region) were calculated based on the bioassay data from the rat oncogenicity study with the high-dose animals removed due to excessive toxicity at this dose level. The unit risk of 2.33×10^{-3} $(mg/kg\ bw/d)^{-1}$ was used for the cancer risk assessment for commercial seed (canola, corn) treatment workers handling Poncho 600 and for workers planting treated seed.

3.5 Impact on human or animal health arising from exposure to the active substance or to impurities contained in it (OECD 2.3.6)

The information in this section is summarized in Appendix II, tables 1 to 10.

3.5.1 Operators

Dermal absorption

In the absence of an in vivo dermal absorption study for clothianidin, a default dermal absorption value of 50% was selected to estimate a systemic dose for the dermal route of exposure based on the available information on the physical-chemical characteristics of the active ingredient and a comparison of oral and dermal toxicities. Clothianidin has a very low log K_{ow} (0.7 at 25°C) and high water solubility (2327 mg a.i./L), which predict a low potential to pass the outermost lipid *stratum corneum* of the epidermis. Its high water solubility indicates any absorbed amounts would have a high potential to dissolve into and pass through the more water-based viable epidermis and into the dermal layer where absorption into the blood would readily occur. Thus, significant skin-bound residues are not anticipated. The USEPA estimated an apparent dermal absorption to be 23%, based on a comparison of NOAELs in the repeat-dosing oral and dermal toxicity studies. Based on a preliminary review of a recently submitted in vivo dermal absorption study conducted in the monkey, it is anticipated that dermal absorption is significantly less than 50% (i.e., approximately 1%).

Use description/exposure scenarios

The proposed registration is for a new end-use product, Poncho 600, for commercial seed treatment of canola and corn. It is a liquid suspension formulation with a guarantee of 48% clothianidin (600 g/L). No on-farm seed treatment by farmer or custom applicator is proposed. The product is packaged in 3.79 or 10 L HDPE jugs or 100 L HDPE drums. The formulation does not require dilution. However, since it does not contain a seed dye; the dye must be added at the time of seed treatment. The proposed application rate is 250 or 666 mL EP/100 kg seed (150 or 400 g a.i./100 kg seed) for canola and 33.3 or 166.7 mL EP/80 000 unit of seed (0.25 or 1.25 mg a.i./kernel) for corn. The draft label specifies handlers must wear a long-sleeved shirt and long pants, waterproof gloves, shoes and socks.

There are two basic types of treaters in common use: a slurry type and a mist type. Both types of equipment have a mixing tank for the preparation of diluted material and a "pump" tank that pumps the diluted mixture from the mixing tank to the seed treatment machine; a treatment area which may be open or closed; and a seed measurement device. The hopper with a "trip weighter" is preset to tip a prescribed weight of seeds into the treatment area. The major difference in the slurry- and mist-type equipment is how the pesticide is applied to the seeds in the treatment area.

In slurry-type equipment, a predetermined volume of the treatment solution is added to the seed as it is released from the hopper. The treatment solution is metered by the same "trip weight" device as the seeds. In mist-type equipment, the process is the same, except that the treatment solution is dumped onto a spinning disc and thrown onto the seed as it passes the edge of the disc. Treated seed then passes to a mixing area, which may be enclosed or have a transparent cover that can be opened. From the treatment area, the seed passes to a holding bin ready for bagging. Seed is usually bagged automatically to a predetermined weight. The bagger then folds the top of the bag, attaches the seed grade certificates and treatment tags, and sews it shut using an automatic sewing machine. Finally, the bag is moved by a conveyer belt to a pallet area where it is stacked for shipping.

Worker activities and numbers of people involved vary at different plants depending on the size of the operation and degree of automation. Usually one worker prepares the treatment slurry (mixer/loader), which involves open transfer of the product into the premix tank for smaller containers and closed transfer for bulk containers. Another worker (often the mixer/loader) oversees the seed treatment area (treater/coater). One or more workers are involved in bagging the seeds as well as sewing, tagging and stacking seed bags. Most seed treatment plant workers have eight-hour shifts and workers may rotate duties to other areas.

Depending on the size of the facility, type of seed treating equipment and type of seed being treated, seed treatment capacity varies (from 20 000 to 100 000 kg seed per day). Average throughputs for canola and corn are considered to be 40 000 and 60 000 kg of seed treated respectively per eight-hour shift. For canola, at the maximum application rate of 400 g a.i./100 kg seed, a worker could handle up to 160 kg a.i./d. For corn, at the maximum application rate of 410 g a.i./100 kg seed (based on the maximum application rate of 1.25 mg a.i./kernel and an average seed weight of 3270 seeds/kg), a worker could handle up to 246 kg a.i./d.

Canola can be treated between October and May, with most seed treatment being carried out between December and April. Corn may be treated from late August through to the end of May. Therefore, there is a potential for an intermediate-term duration of exposure.

Postapplication exposure to the treated seed occurs for farmers at the time of planting. Planting is usually done for approximately one to two weeks in early spring. For canola, at a typical seeding rate of 6 kg/ha, a farmer could plant about 100 ha of canola in a day, handling 600 kg of treated seed. Therefore, at the maximum application rate of 400 g a.i./100 kg seed, a farmer planting treated canola seed could handle 2.4 kg a.i. per day. For corn, at a typical seed rate of 11 to 22 kg/ha, a farmer could plant approximately 30 to 60 ha of corn per day, handling 660 to 1320 kg seed. Therefore, at the maximum application rate of 410 g a.i./100 kg seed, a farmer planting treated corn seed could handle 2.7 to 5.4 kg a.i. per day.

Operator exposure assessment

Estimates of exposure to workers in commercial seed treatment facilities were based on two surrogate passive dosimetry exposure studies that measured the potential dermal and inhalation exposure during seed treatment in commercial facilities:

(i) Exposures of seed-treatment workers to isofenphos during application of Oftanol-containing seed coating to canola seed
(ii) Exposure of workers to triademinol during Baytan 312 FS seed treatment

Both studies are considered applicable to the proposed registration with similar seed treatment facilities and representative activities monitored. The amount of active ingredient handled per day in both studies was significantly less than anticipated for the proposed use.

(i) Summary of Oftanol surrogate study

The purpose of this study was to quantify inhalation and dermal exposure of workers during commercial seed treatment of canola seed with Oftanol (containing isofenphos) and Benlate T. Monitoring was done for isofenphos only. The study was conducted in Niksu, Alberta, Canada, 17–19 January 1989.

Four workers were monitored three times for a total of twelve replicates. Each worker performed a separate task, including mixing/loading, coating, bagging or shift foreman. The mixer/loader prepared the seed coating mix by adding product, dye and water to the mixing tank. The coater operated the open batch blending machine, which involved adding powdered diatomite to the seeds and periodic scraping of the inside of the blender. The bagger attached a bag to the hopper and started the flow of seeds with periodic manual addition of a few extra grams of seed, followed by carrying the bag from the hopper to the sewing station, sewing the bag shut and stacking it onto a pallet. The shift foreman supervised and assisted in the entire operation. All workers wore long-sleeved shirts and pants, coveralls as well as chemical-resistant gloves and respirators. The maximum amount of active ingredient handled per replicate was 92 kg. The average duration of each replicate was 7.4 hours. The major limitation of this study was that only four workers were monitored at one test site, which limits the ability to determine variability due to factors such as facility size, design, operation and work habits.

Dermal exposure was estimated using passive dosimetry. Deposition was measured using dermal patches attached to the inner and outer clothing of each worker. Deposition to the hands was measured using ethanol hand washes. Dermal exposure of covered skin was calculated by extrapolating the inner patch data to standard body surface areas and summing all body area results together with the handwash residues. Dermal exposure of exposed skin (neck and head) was based on the measurements of the outer patches attached to a baseball cap, the chest and the back, which were extrapolated to standard body surface areas. Inhalation exposure was measured using air filters attached to personal air sampling pumps. Total dermal exposure (patch deposition and hands) was added to the inhalation results for each worker and normalized for kg of a.i. handled. Exposure estimates are based on workers wearing a single layer of clothing with gloves.

The mean total unit exposure was highest for the mixer/loader, followed by the shift foreman, the coater and finally the bagger. For the mixer/loader and the coater, the majority of dermal deposition occurred on exposed skin (head and neck). For the bagger and shift foreman, the majority of the exposure occurred on the covered skin. The average contribution of hands to total dermal exposure for all activities was 15% or less. Inhalation exposure accounted for less than 3% in all activities.

To estimate exposure with the addition of cotton coveralls, a 75% protection factor was applied to dermal deposition excluding neck, head and hands. To estimate exposure with the addition of a respirator, a 90% protection factor was applied to inhalation exposure (Appendix II, Table 1).

(ii) Summary of Baytan 312 FS surrogate study

The purpose of this study was to quantify inhalation and dermal exposure of workers during commercial seed treatment of wheat seed with Baytan 312 FS (containing triademinol). Workers were monitored from three Canadian commercial seed treatment facilities in half-day replicates. All workers wore long-sleeved shirts and pants, coveralls and chemical-resistant gloves. The major limitation to this study was the low number of replicates (4 to 12) and the low number of workers who were monitored for a half-day in each replicate.

At the large facility (300 bushels/h capacity), five workers were monitored in half-day replicates over two days. One worker was monitored when the treated seed came from the storage bin into the bagging area. This worker was referred to as the treater/bagger, but his role was mainly operating the bagging machine. Three workers, referred to as stacker/tagger, were monitored when the bags were stacked and tagged. Two of these took the bags from the conveyor belt and stacked them onto pallets. One of these stapled tags to the stacked bags. Each of the three rotated jobs periodically. The fifth worker operated a forklift. The average amount of active ingredient handled per day was 28 kg by the bagger, 13 kg by each stacker/tagger and 36 kg by the forklift operator. The PMRA calculated unit exposures (μg a.i./kg a.i. handled) based on the assumption that the amount handled during the stacking/tagging was shared among the three stacker/taggers.

At the medium facility (200 bushels/h capacity), four workers were monitored in half-day replicates over three days. One worker, referred to as the bagger, was monitored when the treated seed came from the storage bin into the bagging machine, where the worker manually fitted the bag onto the spout. Another worker, referred to as the tagger/sewer was monitored when placing a tag on the bag and when he fed them through a sewing machine. Another two workers were monitored when stacking sewn bags onto pallets. The average amount of active ingredient handled per day was approximately 20 kg a.i./d for the bagger and tagger/sewer, and 10 kg a.i./d for the stackers. The PMRA calculated the unit exposures (μg a.i./kg a.i. handled) for the stackers based on the assumption that the amount handled was shared among the two stackers.

At the small facility (25–40 bushels/h), a single worker was monitored in half-day replicates over three days. The worker, referred to as the treater, performed all tasks of treating seed, bagging (manually fitting bags and topping up with treated seed), tagging, sewing and stacking bags. The average amount of active ingredient handled per day was 1.9 kg by one worker.

In each facility, one additional worker, referred to as the mixer/calibrator, was monitored once during the mixing of the treatment slurry and periodic calibration of the seed treating equipment. Another worker was monitored while disassembling the mix/load equipment. This activity is not considered representative of seed treatment facilities and not discussed further.

Dermal exposure was estimated using passive dosimetry. Deposition was measured using dermal patches attached to the inner and outer clothing of each worker. Deposition to the hands was measured using ethanol hand washes. Dermal exposure of covered skin was calculated by extrapolating the inner patch data to standard body surface areas and summing all body area results together with the handwash residues. Dermal exposure of exposed skin (neck and head) was based on the measurements of the outer patches attached to a baseball cap, the chest and the back, which were extrapolated to standard body surface areas. Inhalation exposure was measured using air filters attached to personal air sampling pumps. Total dermal exposure (patch deposition and hands) was added to the inhalation results for each worker and normalized for kg of a.i. handled. Exposure estimates are based on workers wearing a single layer of clothing with gloves.

For the large facility, the mean total unit exposure was highest for the mixer/calibrator and the treater/bagger. The stacker/tagger and forklift operator exposures were half and 20× less than the treater/bagger, respectively. The majority of dermal deposition occurred on covered skin for all tasks and the contribution from the hands accounted for at most 25%. Inhalation exposures accounted for less than 7% for all tasks.

For the medium facility, the mean total unit exposure was highest for the mixer/calibrator, much higher than the bagger, tagger/sewer and stacker, all of which had similar exposure levels. The majority of dermal deposition occurred on the covered skin and the contribution from the hands accounted for at most 24%. In this facility, inhalation exposure to the bagger and tagger/sewer accounted for a large portion of the total unit exposure, 40 and 52%, respectively.

For the small facility, the mean total unit exposure was highest for the worker who performed all tasks including treating, bagging, sewing and stacking. This worker's exposure was also higher than the mean total unit exposure of the mixer/calibrator. The majority of dermal deposition occurred on the covered skin and the contribution from the hands accounted for less than 8%. Inhalation exposure accounted for 19% of the total unit exposure. For the mixer/calibrator, the contribution from the hands to total dermal deposition was 15% and the contribution from inhalation was only 0.4%. Results are shown in Appendix II, Table 2.

Overall, the highest exposures occurred for the mixer/calibrators, particularly for one individual at the medium facility where high deposition occurred on the right leg. The next highest exposure occurred in the small facility where the individual performed all tasks (3× higher than the next highest exposed, the treater/bagger in the large facility). Inhalation exposures for baggers, taggers, sewers and stackers were relatively high in the medium and small facilities, accounting accounted for, on average, 20% of the total exposure.

To estimate exposure with the addition of cotton coveralls, a 75% protection factor was applied to dermal deposition, excluding the neck, head and hands. To estimate exposure with the addition of a respirator, a 90% protection factor was applied to inhalation exposure.

Risk assessment for seed treatment workers

Overall, the two surrogate studies indicated that the highest unit exposures (μg a.i./kg a.i. handled) in all facilities was for the individual preparing the seed treatment slurry in an open system. The majority of deposition was dermal, with < 1% from inhalation. The next highest unit exposures were for the individual who performed all tasks involved in a small facility including treating, bagging, tagging, sewing and stacking. The majority of deposition was dermal, with a high proportion via inhalation (25%). Unit exposures were comparable for the individuals performing bagging, tagging, sewing and/or stacking, with deposition equally from both dermal and inhalation.

The studies did not provide an estimate of daily exposure for an individual who may perform both the mix/load/calibrate function and the seed treatment function. The studies did not provide an estimate of exposure for an individual performing the mix/load/calibrate functions in a closed system. The studies did not provide an estimate of exposure for an individual involved in clean-up or repair activities.

Exposure and risk estimates were therefore determined for an individual in a typical facility performing combined activities of mixing, loading, calibrating and coating over an intermediate duration of exposure. Unit exposures for all mix/load replicates from both the Oftanol and Baytan 312 FS studies were combined for an overall unit exposure (arithmetic mean) and added to the unit exposure (arithmetic mean) of the coater from the Oftanol study. Because of the wide variability in the exposure estimates for the mix/load/calibrate activities, an acute-duration risk assessment was also performed using the 90th percentile of the mix/load/calibrate replicates.

Exposure and risk estimates were also determined for an individual in a small facility performing all tasks, including mixing, loading, calibrating, treating, bagging, tagging and stacking, for an intermediate duration of exposure. Unit exposures (arithmetic mean) from the small facility were added to the mix/load/calibrate unit exposures (arithmetic mean). Because of the wide variability in exposure estimates for both the mix/load/calibrate activities and the individual monitored in the small facility, an acute-duration risk estimate was also performed using the 90th percentile of the mix/load/calibrate replicates added to the 90th percentile from the treater replicates in the small facility.

Exposure and risk estimates were also determined for individuals in medium to large facilities performing a combination of tasks, including bagging, tagging, sewing and stacking, for an intermediate duration of exposure. Unit exposures (arithmetic mean) from all studies for all tasks of bagging, tagging, sewing and/or stacking were combined for an overall unit exposure (arithmetic mean).

Because dermal exposures were high and many tasks had high inhalation exposures, the addition of coveralls and respirator are recommended. Unit exposures (arithmetic mean) were corrected based on a protection factor of 75% for cotton coveralls and 90% for a respirator. Dermal unit exposures (arithmetic mean) were corrected assuming a default dermal absorption factor of 50% or 1%.

Total systemic exposures were calculated from the equation that follows. Total unit exposures and systemic exposures for seed treatment workers are presented in Appendix II, Table 3.

$$\textit{Total systemic exposure (mg/kg bw/d)} = \frac{\textit{total unit exposure} \times \textit{application rate} \times \textit{kg seeds treated/d} \times 1000\ \mu\textit{g/mg}}{\textit{bw}}$$

where:
total unit exposure = absorbed dermal + inhalation unit exposures (μg a.i./kg a.i. handled)
application rate for canola = 400 g a.i./100 kg seed
application rate for corn = 410 g a.i./100 kg seed
typical kg canola seed treated/d = 40 000 kg
typical kg corn seed treated/d = 60 000 kg
kg seed treated/d (small facility) = 20 000 kg
body weight = 70 kg

For the non-cancer risk assessment, margins of exposure (MOEs) for acute and intermediate durations of exposure were determined with a target MOE of 300 (Appendix II, Table 4).

Based on a default dermal absorption factor of 50%, intermediate-term MOEs would be unacceptable for all seed treatment workers in typical and small facilities. Acute-term MOEs would also be unacceptable for the highest exposed individuals in typical and small facilities.

However, based on a dermal absorption factor of 1%, intermediate-term and acute-term MOEs were acceptable for all seed treatment workers in typical facilities. For a small facility, acute-term MOEs were acceptable and intermediate-term MOEs approached the target MOE of 300.

For the cancer risk assessment, lifetime average daily doses (LADDs) were determined based on a default dermal absorption factor of 50% or 1% according to the equation that follows. LADDs for see workers are presented in Appendix II, Table 5.

LADD = Systemic exposure (mg/kg bw/d) × frequency treating seed/year × no. working years/lifespan

where:
frequency of days treating canola or corn seed in a year = 90days/365 days
no. working years = 40 years
lifespan = 75 years

Cancer risk levels were determined by the following equation and are presented in Appendix II, Table 6.

Risk level = LADD × Q_1^* ***(cancer slope factor), where,*** $Q_1^* = 2.33 \times 10^{-3}\ (\text{mg/kg bw/d})^{-1}$.

Based on a default dermal absorption factor of 50%, risk levels for workers treating canola or corn seed were 20 to 250× higher than target levels of 1×10^{-6}. Based on a dermal absorption factor of 1 %, risk levels would be acceptable provided measures are taken to reduce exposure potential (e.g., closed mix/load systems, a product stewardship programme) and that the effectiveness of these measures are documented (i.e., a chemical-specific exposure study).

3.5.2 Bystanders

The potential for bystander exposure was considered minimal and was significantly less than exposures estimated for operators and workers.

3.5.3 Postapplication exposure

There is a potential for short-term exposure to farmers who plant treated canola or corn seed in early spring for one to two weeks. Estimates of exposure to farmers who plant treated seed were based on surrogate passive dosimetry exposure studies that measured the potential dermal and inhalation exposure during planting of Oftanol-treated canola seeds (*Exposures of workers to isofenphos during planting of Oftanol-treated canola seed*).

Summary of surrogate planting study
The purpose of this study was to quantify inhalation and dermal exposure of workers during the planting of Oftanol-treated canola seed. The study monitored four private growers, each serving as a subject three or four times, for a total of thirteen replicates during loading and planting of canola seed treated with a mixture containing Oftanol (technical isofenphos) and Benlate T. Monitoring and sample analysis was for isofenphos and its oxygen analogue. The study was conducted in Domain, Manitoba, Canada, 16–23 May 1989. Work involved loading the treated seed (25 kg bag) into seed hoppers and planting between 6.7 and 9.0 kg of seed/ha (6–8 lb/acre) using tractor-driven planters.

The duration of each replicate was between 1.83 and 6.24 hours and each worker handled between 0.86 and 2.81 kg of active ingredient per replicate.

Dermal exposure was estimated using passive dosimetry methodology. Dermal deposition was measured using patches attached to the inner and outer clothing of each worker. Deposition to the hands was measured using ethanol hand washes. Potential inhalation exposure was measured using air filters attached to personal air sampling pumps.

Total exposure was estimated for workers wearing a typical seed-planting clothing, including long-sleeved shirt, long pants and chemical-resistant gloves, while handling the treated seed. Total dermal exposure was calculated by extrapolating each interior patch data and two of the exterior patches (upper back and head) to standard body surface areas, and summing results for total body deposition and adding the handwash residues. Inhalation exposure was calculated based on the amount of isofenphos found on the air-sampling filters, the pump flow rate and an assumed respiratory rate of 29 L/min (0.029 m^3/min) for moderate activities. Total exposure was calculated by adding the total dermal exposure and inhalation deposition. Residues were corrected for field recoveries less than 95%, and ½ LOD was used to calculated values reported as < LOD (< LOD values were not corrected for field recovery). Since workers were not monitored for a full work day, results were normalized to μg/kg a.i. handled.

The majority of exposure was dermal, with most occurring on the hands (70%). Inhalation exposure accounted for less than 1% of the total exposure. The mean total unit exposure (body + hands + inhalation) was 425.28 ± 245.79 μg/kg a.i. handled, with values ranging from 183.55 to 947.02 μg/kg a.i. handled.

Risk assessment for workers planting treated seed

For a short-term duration of exposure, risk to workers planting treated seed was determined based on the unit exposure (arithmetic mean) from the surrogate study. Dermal unit exposures were corrected assuming a default dermal absorption factor of 50% or 1%.

For canola, at a typical seeding rate of 6 kg/ha, a farmer could plant about 100 ha of canola in a day, handling 600 kg of seed. At the maximum application rate of 400 g a.i./100 kg seed, a farmer planting canola seed could handle 2.4 kg a.i. per day. For corn, at a typical seed rate of 11 to 22 kg/ha, a farmer could plant approximately 30 to 60 ha of corn per day, handling 660 to 1320 kg seed. At the maximum application rate of 410 g a.i./100 kg seed, a farmer planting corn seed could handle 2.7 to 5.4 kg a.i. per day.

Dermal exposure was calculated from the following equation and are presented in Appendix II, Table 7.

Dermal exposure (mg/kg bw/d) = unit exposure × amount handled per day ÷ bw

where:
kg a.i. handled/day planting canola = 2.4 kg
kg a.i. handled/day planting corn = 5.4 kg
body weight = 70 kg

For the non-cancer risk assessment, MOEs for short-term duration of exposure were determined (Appendix II, Table 8). Based on the exposure estimate from the surrogate study, target MOEs were achieved for farmers planting treated canola or corn seed wearing a long-sleeved shirt, long pants and chemical-resistant gloves, assuming a default dermal absorption of 50%. It should be noted that unit exposure estimates from the submitted surrogate study may be underestimated by as much as 100×, based on knowledge of this exposure scenario. However, at a dermal absorption of 1%, MOEs are adequate to compensate for potential exposures of 100× higher.

For the cancer risk assessment, the lifetime average daily doses (LADDs) were calculated by the following equation and are presented in Appendix II, Table 9.

LADD = Daily systemic exposure (mg/kg bw/d) × frequency of planting seed/year × no. working years ÷ lifespan

where:
frequency of planting canola/corn seed/year = 10/365 days
no. working years = 40
lifespan = 75

Cancer risk levels were determined by the following equation and are presented in Appendix II, Table 10.

Risk = LADD × Q_1^* ***(cancer slope factor)***, where $Q_1^* = 2.33 \times 10^{-3}$ **(mg/kg bw/d)**$^{-1}$.

In the cancer risk assessment, risk levels for workers planting treated seed achieved target levels of 1×10^{-6}, based on a default dermal absorption factor of 50%. It should be noted that unit exposure estimates from the submitted surrogate study may be underestimated by as much as 100×, based on knowledge of this exposure scenario. However, at a dermal absorption of 1%, risk levels are acceptable for potential exposures of 100× higher.

4.0 Residues

4.1 Food residue summary

Analytical methodology in plant matrices

Acceptable method validation has been submitted for the LC-MS/MS method (Method 00552-M001) proposed for enforcement of tolerances of TI-435 in/on plant commodities. Validation data were included for determination of the parent compound, using external bracketing standards and internal standard quantitation. A successful ILV has been completed with corn grain. Satisfactory radiovalidation data have been submitted for apple, corn forage, stover and grain. An interference study has been conducted with 133 compounds, and no interferences were observed. The LOQ, using internal standard, is 0.01 ppm, with the exception of wheat straw (0.02 ppm).

Analytical methodology in animal matrices

Acceptable method validation has been submitted for the LC-MS/MS method (Method 00624) proposed for enforcement of tolerances of TI-435 in ruminant commodities. Validation data were included for the metabolites TZG, TZU and ATMG-pyruvate, as well as for the parent compound. A successful ILV has been completed with liver, satisfactory radiovalidation data have been submitted for milk, muscle, fat and liver, and no interference has been observed in a study that investigated potential interferences from 163 compounds. Radiovalidation provided indicate that the metabolite ATMG-pyruvate is not adequately extracted from bioincurred fat samples previously stored in a freezer from the metabolism study. The LOQ is 0.01 ppm for each analyte in milk and 0.02 ppm for each analyte in animal tissues.

Nature of the residue in animals

Laying hens were orally dosed for three consecutive days, in time intervals of 24 hours, with [nitroimino-^{14}C] TI-435 at a dose level of 10 mg/kg body weight and were sacrificed five hours after the last dosage. More than 94% of the administered dose was excreted within 53 hours after the first administration with no tissue or organ exceeding the equidistribution concentration of 10 μg/g. Major metabolites (>10% of the total radioactive residues [TRRs]) identified in eggs were TZNG and TI-435. The predominant residues in tissues were TZNG in fat and liver, ATG-Ac in muscle and fat, and TZG in liver. Minor metabolites in eggs and tissues were TI-435, TZNG, ATMT, MNG, TZG, TMT, ATG-pyruvate, TZU, MNG, NTG, TMG and urea identified each at less than 10% of the TRRs. The parent compound, clothianidin, is the residue of concern for enforcement of the maximum residue limit. Clothianidin, TZNG, TZU, TZG and ATMG-pyruvate are the residues of concern to be included in the risk assessment.

A lactating goat was dosed orally by intubation with [nitroimino-^{14}C] TI-435, at a dose level of 9.8 mg/kg bw/d for three consecutive days and was sacrificed 53 hours after the first dose administered. The total radioactive residue in edible tissues and organs represented approximately 6.6% of the administered dose and 1.5% in milk. Of the 70.4% recovered, the remaining 30% of the administered dose was assumed to be present in the contents of the gastrointestinal tract at sacrifice (53 hours after the first administration). Major metabolites (>10% of the TRRs) identified included TI-435 and TZU in muscle; TZMU, TZU, TZG and ATMG-Pyr in kidney; TMG-adducts in liver; TI-435, TZMU and TZU in fat; as well as TI-435, TZNG and TZU in milk. Minor metabolites (<10% of the TRRs) identified in muscle, kidney, fat and eggs included TZNG, TZMU, TZU, TMG, MG, MNG, NTG, TZG, ATMG-Pyr, TMHG and urea. The parent compound, clothianidin, is the residue of concern for enforcement of the maximum residue limit. Clothianidin, TZNG, TZU, TZG and ATG-acetate are the residues of concern to be included in the risk assessment.

Nature of the residue in plants
Nitroimino labelled TI-435 was applied to corn (1.06 mg a.i./seed) and sugar beet (190 g a.i./ha) as seed treatment, to apple by foliar application (2 × 202 g a.i./ha), to tomato by soil (15 mg a.i./plant) and foliar (2 × 157.8 g a.i./ha) application. Thiazolyl labelled TI-435 was applied to corn seed at a rate of 2.52 mg a.i./seed. Major metabolites (>10% of the TRRs) identified included TI-435 and TZMU (apple), TI-435 and MNG (tomato), TI-435, MG and TMG (sugar beet), and TI-435 and MG (corn). However, TZMU had an absolute value of 0.009 ppm in apple fruit. There is no reason to believe that TZMU is any more toxic than the parent. TMG was also a rat metabolite and it was considered unlikely that TMG would contribute to the toxicological effects seen in the rat study. Minor metabolites (<10% of the TRRs) were also identified and included TZNG (corn, sugar beet, apple and tomato), MG (corn, sugar beet and apple), MNG (corn, sugar beet and apple), NTG (corn, sugar beet, apple), THMN-Glc (apple), TMG (corn, sugar beet, apple), TZU (corn, sugar beet and apple), TZMU (corn, sugar beet) and CTCA (corn). Therefore, for the purpose of risk assessment and enforcement of the maximum residue limit, only the parent compound (TI-435) is considered the residue of concern.

Confined rotational crops
Total radioactive residues accumulated at ≥ 0.01 ppm in all commodities of turnip, Swiss chard, and wheat planted 29, 153 and 314 days after a single soil application of TI-435 at 328 g a.i./ha (0.293 lb a.i./acre). Radioactivity was lowest in turnip roots and wheat grain and highest in wheat straw; TRRs generally decreased at longer plantback intervals (PBIs). Approximately 65.9–98.2% of the TRRs were extracted from rotational crop matrices using acetonitrile and water; for wheat grain and straw, microwave extraction was used to release additional bound residues. Furthermore, the 314-day wheat grain samples were subjected to acid hydrolysis with 2 N HCl at reflux. Total residues amounting to 31.0–86.0% of the TRRs were identified in rotational crop matrices from all PBIs. In general, the parent TI-435 and the metabolites TZNG and MNG were the predominant residues in rotational crops from all PBIs. Additional identified metabolites included TZMU, NTG, TZU, TMG and MG. Non-extractable residues following extraction and hydrolysis procedures accounted for 1.8–20.3% of the TRRs (<0.001–0.211 ppm). Based on the submitted data, the analytical methods used were considered acceptable to determine the identity of major components in rotational crops. Clothianidin is the residue of concern for enforcement of the maximum residue limit.

Clothianidin, TZNG and MNG are the residues of concern to be included in the risk assessment.

Field accumulation in rotational crops

Residues of TI-435 were observed in mustard greens, turnip tops, wheat forage and wheat hay at all PBIs except the 12-month PBI. The maximum TI-435 residues occurred at the eight-month PBI for mustard greens, turnip tops and wheat forage and the one-month PBI for wheat hay. Residues were below the LOQ (<0.01 ppm) at all PBIs for turnip roots, wheat straw and wheat grain. Residues of TZNG were below the LOQ (<0.01 ppm) for all rotational crops at the one-month PBI. Therefore, it is recommended that leafy, root and tuber vegetables have a plantback restriction of one year and cereal grains, grasses, non-grass animal feeds, soybeans and dried beans have a plantback restriction of 30 days. Corn and canola seed may be replanted immediately.

Supervised residue trials

Supervised residue trials were conducted on corn and canola as seed treatments. The residue data indicate that clothianidin residues were less than 0.01 ppm in grain and sweet corn (the corn commodities used directly for human consumption) after the use of Poncho 600 at 2.0 mg a.i./seed. The highest individual values of the clothianidin residue in the livestock feed items (early milk-stage corn forage with ears, late dough-stage corn forage and fodder) was in the range of 0.037 to 0.061 ppm. In three decline trials (Tifton, Oxford, Branchton), clothianidin residue in early milk-stage corn forage with ears (0.016–0.022 ppm), corn fodder (0.010 ppm) and grain (<0.010 ppm) declined slightly or remained constant with time (within 20 days). The use of Poncho 600 at 600 g a.i./100 kg seed (39.9 to 43.3 g a.i./ha), resulted in residues of clothianidin on canola seed at less than 0.01 ppm.

Storage stability

Samples of sugar beet (body), corn (grain, forage, straw), and canola (seed) spiked with clothianidin at a level of 0.2 mg/kg were stored at -18°C for intervals of 1 month, 3 months, 6 months, 12 months, 18 months and 24 months. Under these conditions, residues of TI-435 did not decrease by more than 16% in the matrices evaluated after 24 months. No corrections to residue values due to in-storage dissipation are necessary.

Processing studies

Following exaggerated use rates of Poncho 600 on corn (10 mg a.i./seed) and canola (2400 mg a.i./100 g seed), no TI-435 residues greater than the LOQ of 0.01 ppm were observed in the corn grain (raw agricultural commodity) or in the canola seed. Since no seed heads were present when Poncho 600 was applied as a seed treatment to corn, no analyses of aspirated grain fractions were conducted. A waiver of the requirement to conduct analyses of the aspirated corn grain fractions is acceptable.

Livestock feeding

No quantifiable residues above the LOQ (0.01–0.02 ppm) of any analyte (TI-435, TZG, TZU and ATMG-pyruvate) were found in tissues. With the exception of the parent clothianidin at the highest dose, no quantifiable residues were found in milk. At the highest dose level, 2.56 mg/kg of feed, residues of clothianidin could be quantified in milk, ranging from < 0.01 ppm to 0.012 ppm.

Dietary risk assessment

The proposed domestic use of clothianidin (Poncho 600) on corn and canola does not pose an unacceptable chronic or acute dietary (both food and water) risk to any segment of the population, including infants, children, adults and seniors. In view of the fact that the estimated exposure from drinking water is conservative, the lifetime cancer risk estimates (both food and water) are still within the level of concern.

5.0 Fate and behaviour in the environment

See Appendix V, Environmental assessment, for summary tables.

5.1 Physical and chemical properties relevant to the environment

Clothianidin is very soluble in water. This is one of the indicators of high potential to leach. The vapour pressure of clothianidin is 1.3×10^{-10} Pa at 25°C, and Henry's Law constant calculated by the reviewer is 9.8×10^{-16} atm m^3/mole. These values indicate that clothianidin is non-volatile from water and moist soil surfaces. The log K_{ow} is 0.7. This indicates that clothianidin has low potential to bioaccumulate. Based on the pK_a values, clothianidin will not dissociate in acidic and neutral solutions. The maximum light absorption was at 265.5 nm in neutral solution, indicating minimal potential for phototransformation under natural environment.

5.2 Abiotic transformation

Clothianidin was stable to hydrolysis during the 30-day study period. Therefore, it is not considered to be an important route of transformation in the environment. No major or minor transformation products were identified at pH 5 and pH 7. Minor transformation products identified at pH 9 were CTNU and TZMU. Data on the phototransformation of clothianidin on soil and in water are not required for seed treatment use. As clothianidin is non-volatile under field conditions, no data on the phototransformation in air are required.

5.3 Biotic transformation

Clothianidin is very persistent in soil under aerobic conditions, with a first-order half life of 495 to 990 days. The major transformation products identified were TZNG and MNG with a maximum concentration of 10 to 11% of applied radioactivity. Volatile CO_2 formation reached a maximum of 17% of applied. The minor transformation products formed in aerobic soil included TZMU, MNG and NTG. Aerobic biotransformation of MNG and TZNG in soil indicated that they are moderately persistent with half-lives of 87 to 115 days for MNG, and 71 to 124 days for TZNG.

Biotransformation will be a route of dissipation for clothianidin under aerobic conditions in the terrestrial environment.

5.4 Mobility

The adsorption and desorption studies indicated that clothianidin has moderate to high mobility in soil. The adsorption K_d values were 0.52–4.14, and the adsorption K_{oc} values ranged from 84 to 345, indicating high to moderate mobility. Based on the adsorption K_{oc} values, MNG and TZMU have high to very high mobility in soil, TZNG has moderate mobility, and TMG is immobile to low mobility in soil. These results indicate that the parent and some of the transformation products (MNG, TZNG, TZMU) have high to moderate potential for leaching, and TMG has low potential to leach. The high to moderate potential for leaching of clothianidin is also supported by its high water solubility.

5.5 Dissipation and accumulation under field conditions

Five studies were submitted on soil dissipation/accumulation of TI-435 under Canadian, American and German field conditions. The three Canadian studies were conducted on bare plots in Ontario (Ecoregion 8.1, mixed wood plains) and Saskatchewan (Ecoregion 9.3, west central semi-arid prairies). One American field study conducted in a bare plot in North Dakota comes under U.S.–Canada common Ecoregion 9.2 (temperate prairies); therefore, it represents Canadian use conditions. The other two American field studies were conducted in bare plots in Ohio and in Wisconsin (Ecoregion 8.2, central U.S. plains). Two supplemental field lysimeter studies were conducted in Germany. For the Canadian and American studies, TI-435 FS600, a formulation product with 595.1 g a.i./L, was used.

Under Canadian field conditions, TI-435 dissipation in the 0–15 cm soil depth was slow. Clothianidin residues were not detected below the 30–45 cm soil depth. The DT_{50} values at the Ontario and North Dakota sites were 385 and 1386 days, respectively. A DT_{50} for the Saskatchewan study could not be determined due to limited dissipation during the study. TI-435 concentration at this site at the end of 775-day period was, however, 80% of the 0-day concentration. These values indicate that TI-435 is persistent in soils under Canadian use conditions. No major transformation products were detected at any of the test sites. Four minor transformation products, TZNG, MNG, TZMU and TMG, were detected under field conditions.

At all relevant Canadian sites, parent compound and its transformation products were not detected below the 30 or 45 cm soil depth. These results indicate that TI-435 and its transformation products have a low potential for leaching beyond 45 cm under these field study conditions. The laboratory studies, however, indicated a moderate to high mobility of clothianidin. It should be noted that the field studies were conducted with only one time application of clothianidin. Based on the persistence and laboratory studies of mobility, it is possible that clothianidin residues would move to lower soil depths when applied continuously for multiple years. This may result in ground water contamination, particularly in areas where the water table is shallow.

The DT_{90} values at the Ontario and North Dakota sites were 1279 and 4606 days, respectively. A DT_{90} for the Saskatchewan study could not be determined due to limited dissipation during the study. The residue carry-over at the end of a 4- to 6-month period (canola and corn season) was > 80% of the 0-day concentration in field conditions comparable to Canadian use areas. These results indicate that TI-435 carry-over to the following season is very high.

The DT_{50} values for the Ohio and Wisconsin sites were 315 and 408 days, respectively. The corresponding DT_{90} values were 1047 and 1355 days.

5.6 Bioaccumulation

The low K_{ow} indicates that clothianidin has low potential to bioaccumulate in organisms.

5.7 Summary of fate and behaviour in the terrestrial environment

Various laboratory transformation and field dissipation studies indicated that clothianidin (TI-435) is persistent in the terrestrial environment. Hydrolysis of clothianidin was minimal under acid and neutral pH conditions. Based on the continuous irradiation, the first-order half-lives of clothianidin were 8.2 d and 182 d in the irradiated and dark control soils. The aerobic biotransformation of clothianidin indicated that it is persistent in soil. The major transformation products formed under aerobic soils are TZNG and MNG. The minor transformation products included TZMU and MTG. Adsorption/desorption studies indicated that clothianidin has moderate to high mobility in soil; MNG has very high mobility; TZMU has very high to high mobility; TZNG has moderate mobility; and TMG has low mobility or is immobile in soil. Field dissipation studies indicated high persistence and potential residue carry-over of the applied clothianidin into the following growing season. Most of the residues remained in the 0–30 cm or 0–45 cm soil depths in the relevant Canadian field sites. It should, however, be noted that these field studies were conducted with only one year application of clothianidin. Based on the persistence and laboratory studies of mobility, it is possible that clothianidin residues will move to lower soil depths when applied continually for multiple years. This may result in ground water contamination, particularly in areas where the water table is shallow. No major transformation products were detected in field studies. The minor transformation products detected under field conditions included TZNG, MNG, TZMU and TMG.

5.8 Expected environmental concentrations

5.8.1 Soil

The maximum application rate of Poncho 600 (600 g a.i/L) is for corn. It is applied at 1.25 mg a.i/kernel. Based on a seeding rate of 84 000 kernels/ha, the maximum application rate is 105 g a.i/ha. The expected environmental concentration (EEC) in soil (15 cm soil depth) using this application rate is 0.047 mg a.i/kg soil, based on a bulk density of 1.5 g/cm^3.

5.8.2 Aquatic systems

Drinking water

Clothianidin residues in potential drinking water sources (ground water and surface water) were modelled at Level 1 (Screening Level) and Level 2. The maximum drinking water concentration of clothianidin in groundwater as a result of leaching was estimated using the LEACHM model (maximum annual peak over 20 years; Appendix V, Table 4). Drinking water concentrations in surface water sources (reservoir and dugouts) as a result of surface run-off were estimated using the linked PRZM/EXAMS models (90th percentile of the yearly peak and yearly average over 50 to 75 years; Appendix V, Table 4). These values are considered to be "upper bound" concentrations in a drinking water source.

For the Level 2 modelling, the model was run using the environmental profile of clothianidin and using an application rate of 105 g a.i./ha applied once. The estimated environmental concentrations of clothianidin in drinking water sources are 23.71 μg a.i./L and 16.48 μg a.i./L (acute for Ontario and Quebec, respectively), and 22.13 μg a.i./L and 13.23 μg a.i./L (chronic for Ontario and Quebec, respectively) (Appendix V, Table 5).

5.8.3 Food sources (grain and seeds)

The maximum expected environmental concentration on food sources such as grain and seeds can be calculated based on the rate of treatment of canola and corn seed for planting.

The rate of application (seed treatment) of clothianidin is a maximum of 400 g a.i./100 kg canola and rapeseed. According to the *Canola Growers Manual* (www.canola-council.org), one kilogram of *Brassica napus* (Argentine canola) contains about 250 000 seeds. The mass of each seed would be approximately 4 mg. Given that the rate of treatment of canola seed is proposed to be 4 g a.i./kg seed, each treated canola seed will have approximately 4 ÷ 250 000 = 0.000016 g a.i. (≡ 16 μg a.i. per seed).

For corn, the rate of application (seed treatment) of clothianidin is a maximum of 1.25 mg a.i. per kernel.

6.0 Effects on non-target species

6.1 Effects on terrestrial organisms

Clothianidin was determined to be highly toxic to the honey bee, *Apis mellifera*, on an acute oral basis with a LD_{50} of 0.00368 μg/bee. The transformation products TMG, MNG, and TZMU were determined to be of relatively lower toxicity to the bee, with an acute oral LD_{50} of > 152 μg/bee, > 153 μg/bee, > 113 μg/bee, respectively. The transformation product TZNG was determined to be of moderate toxicity to the bee, with a LD_{50} of 3.95 μg/bee. Field or semi-field studies conducted in Sweden, the United Kingdom, France and Germany as well as in Ontario (Canada) and Minnesota (United States) indicated that there were no significant impacts on honey bees compared with the controls. All of the field/semi-field studies, however, were found to be deficient in design and conduct of the studies and were, therefore, considered as supplemental information only. Furthermore, the results of most of these studies indicated that residues of clothianidin, when used as a canola (rapeseed) seed treatment insecticide, were expressed in pollen and nectar of the crop plants (or collected from foraging bees). While these residues are not likely to cause acute mortality or other short-term effects, questions remain about the possibility of long-term effects on honey bee colonies. A chronic, multigeneration field study has been requested to clarify this risk.

Clothianidin was practically non-toxic to the bobwhite quail, *Colinus virginianus*, but moderately toxic to the Japanese quail, *Coturnix coturnix japonica*, when administered orally, with an acute LD_{50} of > 2000 mg a.i./kg and 423 mg a.i./kg, respectively. The corresponding no observed effect levels (NOELs) were 500 mg a.i./kg and 12.5 mg a.i./kg. Clothianidin was practically non-toxic to the bobwhite quail and the mallard duck, *Anas platyrhynchos*, on an acute dietary basis, with a LD_{50} of > 5230 mg a.i./kg diet and > 5040 mg a.i./kg diet, respectively. The corresponding no observed effect concentrations (NOECs) for the two avian species were 309 mg a.i./kg diet and 646 mg a.i./kg diet.

In a one-generation reproduction study with bobwhite quail and mallard duck, the NOECs were determined to be 205 mg a.i./kg diet for both species. Significant treatment-related effects included eggshell thinning in the bobwhite quail and decreased number of eggs laid in the mallard duck. Results from the feeding studies conducted with the Japanese quail were inconclusive while results in the domestic pigeon, *Columba livia f. domestica*, indicated that there was decreased feeding activity and possible aversive response to clothianidin.

Clothianidin was determined to be of low toxicity to rats when administered as a single dose via the oral route ($LD_{50} > 5000$ mg/kg bw). The clinical symptoms in dosed rats included ataxia, tremor, palpebral closure and hunched posture. Clothianidin, however, was determined to be highly toxic to mice when administered as a single dose via the oral route (LD_{50} 389 mg/kg bw for males and 465 mg/kg bw for females). The clinical symptoms in dosed mice included decreased activity, ataxia, tremor, palpebral closure, lethargy, prone posture, twitching and hypothermia. Clothianidin was of low toxicity to rats when administered via the dermal route ($LD_{50} > 2000$ mg/kg bw). There were no clinical signs of toxicity in the test animals and no abnormal observations at necropsy. Clothianidin was of low toxicity to rats when administered by the inhalation route ($LC_{50} > 5.53$ mg/L). Clinical symptoms included lethargy, ataxia, semi-closed eyes and hunched body posture. Clothianidin was found to be non-irritating to the skin and slightly irritating to the eyes of rabbits, as well as non-sensitizing to the skin of guinea pig.

Repeated short-term oral dosing of clothianidin to Beagle dogs resulted in lower food consumption, lower body-weight gain, anemia, haemorrhage as well as a decline in leucocytes, lymphocytes and neutrophils (NOAEL: 34.3 mg/kg bw/d for males and 35.8 mg/kg bw/d for females). Oncogenicity studies with mice and rats indicated increased incidence of pulmonary congestion, adrenal cortex congestion, cervix fibromuscular hyperplasia, pelvic mineralization, transitional cell hyperplasia, stomach edema, altered hepatocellular eosionphilic focus and thyroid follicular cysts (NOAEL: 65.1 and 156.5 mg/kg bw/d, respectively). There was, however, no evidence of treatment-related oncogenicity. Clothianidin was considered to be unlikely to present a genotoxic hazard and was non-mutagenic in a standard battery of genotoxicity and mutagenicity tests such as bacterial reverse mutation (Ames test), mammalian gene mutation, and mammalian cytogenetics (micronucleus assay), but showed a positive response in the Chinese hamster lung cell study for chromosomal aberration in vitro. Clothianidin was not neurotoxic to rats and non-teratogenic, but was a reproductive toxicant to rats and rabbits.

In a multi-generation reproduction study with rats (effects on pregnancy and fetuses), clothianidin caused a decrease in body weight and body-weight gain, decrease in thymus weight, increase in stillbirths, delayed sexual maturation, and decrease in spleen weight (NOAELs: 31.2 mg/kg bw/d for parental systemic toxicity, and 9.8 mg/kg bw/d, for offspring toxicity).

6.2 Risk characterization

6.2.1 Environmental behaviour

Clothianidin is very persistent in soil under aerobic conditions, with the formation of two major transformation products: TZNG and MNG. These major transformation products are moderately persistent in soil. Field studies of dissipation and accumulation with clothianidin also confirm its high persistence in soil as well as a high potential for carry-over into the succeeding growing season. Clothianidin has a moderate to high potential for mobility in soil based on adsorption studies conducted in the laboratory. TZNG has a moderate potential for mobility in soil, whereas MNG has a very high potential for mobility in soil in laboratory studies. Under field conditions, however, clothianidin and the major transformation products TZNG and MNG were not detected below the 30–45 cm depth of soil, indicating that these compounds may be less mobile under field conditions than laboratory studies indicated.

6.2.2 Terrestrial organisms

6.2.2.1 Non-target terrestrial invertebrates

Clothianidin is very highly toxic to honey bees, with a 48-hour acute oral LD_{50} of 0.00368 μg a.i./bee (= 3.68 ng a.i./bee). The transformation products TMG, MNG, TZMU and TZNG, however, were of relatively lower toxicity to the honey bees. Field or semi-field studies conducted in Sweden, the United Kingdom, France and Germany as well as in Ontario (Canada) and Minnesota (United States) indicated that there were no significant impacts on honey bees compared with the controls. All of the field/semi-field studies, however, were found to be deficient in design and conduct of the studies and were, therefore, considered as supplemental information only. Moreover, in most of these studies, the results indicated that residues of clothianidin, when used as a canola (rapeseed) seed treatment insecticide, were expressed in pollen and nectar of the crop plants (or collected from foraging bees). While these residues are not likely to cause acute mortality or other short-term effects, questions remain about the possibility of long-term effects on honey bee colonies. A chronic, multigeneration field study has been requested to clarify this risk. It should also be noted that clothianidin is very persistent in soil, with high carry-over of residues to the next growing season. Clothianidin is also mobile in soil.

Given the foregoing, the risk that clothianidin seed treatment may pose to honey bees and other pollinators cannot be fully assessed, owing to the lack of sufficient information and data. Clothianidin may pose a risk to honey bees and other pollinators, if exposure occurs via pollen and nectar of crop plants grown from treated seeds.

6.2.2.2 Wild birds

Corn seed treatment

Dietary and reproductive risk to wild birds from corn seeds treated with Poncho 600

Wild birds, such as bobwhite quail and mallard duck, could be exposed to clothianidin residues as a result of consuming treated seeds.

The bobwhite quail diet consists of 55% seeds (USEPA, 1993). Since the seeds would be treated at the rate of 1.25 mg a.i./kernel of corn (= 7.5 g a.i./kg seed), the estimated ingestion of clothianidin residues can be estimated. The bobwhite quail (live weight 170 grams) consumes the equivalent of 8.94% of its body weight in food daily (Urban and Cook, 1986), 55% of which are seeds. Therefore, the bird would ingest a dose of the active ingredient as follows:

$$(0.089 \times 170) \times 0.55 \times 7500 \div 1000 = 62.4 \text{ mg a.i./d}$$
$$\text{equivalent to } (1000 \div 170) \times 62.4 = 367.05 \text{ mg a.i./kg bw/d}$$

This value is higher than the acute dietary NOEC (27.6 mg a.i./kg bw/d) and reproductive NOEC (18.3 mg a.i./kg bw/d) for the bobwhite quail. The calculated margins of safety for this species are 0.07 and 0.05, respectively. Clothianidin, therefore, poses a high risk to the bobwhite quail on a dietary and reproductive basis.

The diet of mallard duck consists of 70% grain/seeds (USEPA, 1993). Since the corn seeds would be treated at the rate of 7.5 g a.i./kg seed, the estimated ingestion of clothianidin residues can be calculated. The mallard duck (live weight 1.2 kg) consumes equivalent to 4.17% of its body weight in food daily (Urban and Cook, 1986), 70% of which are seeds. Therefore, the bird would ingest a dose of the active ingredient as follows:

$$(0.041 \times 1200) \times 0.70 \times 7500 \div 1000 = 258.3 \text{ mg a.i./d}$$
$$\text{equivalent to } (1000 \div 1200) \times 258.3 = 215.25 \text{ mg a.i./kg bw/d}$$

This value is higher than the acute dietary NOEC (26.9 mg a.i./kg bw/d) and the reproductive NOEC (8.5 mg a.i./kg bw/d) for the mallard duck. The calculated margins of safety for this species are 0.12 and 0.04, respectively. Clothianidin, therefore, poses a moderate risk on a dietary basis and a high risk on a reproductive basis to the mallard duck.

Acute risk to wild birds from corn seeds treated with Poncho 600

According to the proposed label for Poncho 600, corn seeds would be treated at the rate of 1.25 mg a.i./seed of corn.

The NOEL, based on sublethal toxicity, for the Japanese quail was reported to be 12.5 mg a.i./kg body weight. To reach this concentration, the Japanese quail would have to consume 12.5 ÷ 1.25 mg a.i. per seed = 10 seeds/kg bw. However, the Japanese quail weighs approximately 170 grams. Therefore, the amount of active ingredient required to reach the NOEL is 2.12 mg/bird. This dose would be acquired from
2.12 ÷ 1.25 = ~ 1.7 seeds.

Given that each kernal (seed) of corn weighs ~ 160 mg and the daily feed consumption by the Japanese quail is 8.35 g, the estimated consumption of treated seeds by this bird would be 52 seeds per day. The calculated margin of safety based on the number of seeds that would be ingested by the bird is 0.03.

Clothianidin, therefore, poses a high risk to wild birds on an acute basis when used for seed treatment of corn.

Canola seed treatment

Dietary and reproductive risk to wild birds from canola seeds treated with Poncho 600

Wild birds, such as bobwhite quail and mallard duck, could be exposed to clothianidin residues as a result of the consumption of treated seeds.

The bobwhite quail diet consists of 55% seeds (USEPA, 1993). Since the seeds would be treated at the rate of 400 g a.i./100 kg canola seed (= 4 g a.i./kg seed), the estimated ingestion of clothianidin residues can be estimated. The bobwhite quail (live weight 170 grams) consumes the equivalent of 8.94% of its body weight in food daily (Urban and Cook, 1986), 55% of which are seeds. Therefore, the bird would ingest a dose of the active ingredient as follows:

$$(0.089 \times 170) \times 0.55 \times 4000 \div 1000 = 33.28 \text{ mg a.i./d}$$
$$\text{equivalent to } (1000 \div 170) \times 33.28 = 195.76 \text{ mg a.i./kg bw/d}$$

This value is higher than the acute dietary NOEC (27.6 mg a.i./kg bw/d) and the reproductive NOEC (18.3 mg a.i./kg bw/d) for the bobwhite quail. The calculated margins of safety for this species are 0.14 and 0.09, respectively. Clothianidin, therefore, poses a moderate risk on a dietary basis but a high risk on a reproductive basis to the bobwhite quail.

The diet of mallard duck consists of 70% grain/seeds (USEPA, 1993). Since the seeds would be treated at the rate of 4 g a.i./kg seed, the estimated ingestion of clothianidin residues can be calculated. The mallard duck (live weight 1.2 kg) consumes the equivalent of 4.17% of its body weight in food daily (Urban and Cook, 1986), 70% of which is seeds. Therefore, the bird would ingest a dose of active ingredient as follows:

$$(0.041 \times 1200) \times 0.70 \times 4000 \div 1000 = 137.76 \text{ mg a.i./d}$$
$$\text{equivalent to } (1000 \div 1200) \times 137.76 = 114.8 \text{ mg a.i./kg bw/d}$$

This value is higher than the acute dietary NOEC (26.9 mg a.i./kg bw/d) and the reproductive NOEC (8.5 mg a.i./kg bw/d) for the mallard duck. The calculated margins of safety for this species are 0.23 and 0.07, respectively. Clothianidin, therefore, poses a moderate risk on a dietary basis and a high risk on a reproductive basis to the mallard duck.

Acute risk to wild birds from canola seeds treated with Poncho 600

According to the *Canola Growers Manual* (www.canola-council.org), one kilogram of Argentine canola *(Brassica napus)* contains about 250 000 seeds. The mass of each seed would be approximately 4 mg. Given that the rate of treatment of canola seed is proposed to be 4 g a.i./kg seed, each treated canola seed will have approximately $4 \div 250\,000 = 0.000016$ g a.i. ($\equiv 16\ \mu$g a.i. per seed).

Based on sublethal toxicity, the NOEL for the Japanese quail was reported to be 12.5 mg a.i./kg body weight. To reach this level, the Japanese quail would have to consume 12.5 ÷ 0.016 mg a.i./seed = 781.2 seeds/kg bw. Given that the Japanese quail weighs 170 grams, the amount of active ingredient required to reach the NOEL is 2.12 mg/bird. The Japanese quail would acquire this dose from 2.12 ÷ 0.0016 = 1325 seeds.

Given that each *B. napus* seed weighs 4 mg and the daily feed consumption of the Japanese quail is 8.35 g, the estimated consumption of treated seeds by the species would be 2087 seeds. The calculated "margin of safety" based on the number of seeds that would be ingested by the bird is 0.63.

Clothianidin, therefore, poses a moderate risk to wild birds on an acute basis when used for seed treatment of canola.

It should be noted, however, that smaller birds are likely to be at greater risk than the indicator species considered in this risk assessment for corn and canola seeds treated with clothianidin owing to the relatively higher exposure levels expected per unit of body mass of small sized birds.

6.2.2.3 Wild mammals

The most likely route for exposure to Poncho 600 for wild mammals would be through consumption of corn or canola seeds treated with clothianidin insecticide. The acute risk for wild mammals is presented in Table 6.2.2.3.1.

For purposes of this assessment, the acute oral LD_{50} of clothianidin to mice (389 mg a.i./kg body weight) is used. The clinical symptoms in dosed mice included decreased activity, ataxia, tremor, palpebral closure, lethargy, prone posture, twitching and hypothermia. Since data on the toxicity of clothianidin to wild mammals were unavailable, the mouse acute oral LD_{50} was used as a surrogate endpoint for small, medium and large wild mammals.

Assuming that a small mammal (body weight 0.015 kg), medium mammal (body weight 0.035 kg) and a large mammal (body weight 1 kg), each consumes food at 14.6%, 12.5% and 6.9%, respectively, of their body weight per day (Nagy, 1987), the estimated food consumption by each mammal would be as follows:

small mammal	0.015 kg × 14.6% = 0.0022 kg/d (= 146.6 g/kg bw/d)
medium mammal	0.035 kg × 12.5% = 0.0044 kg/d (= 125.7 g/kg bw/d)
large mammal	1.0 kg × 6.9% = 0.069 kg/d (= 69.0 g/kg bw/d)

Table 6.2.2.3.1 **Acute risk from corn or canola seed treated with Poncho 600**

Seed	Mammal size	Calculation	Dose acquired (mg a.i./kg bw/d)	Consumption required (seeds)
Corn [1]	small	(146.6 g/kg bw/d ÷ 0.16 g seed weight) × 1.25 mg a.i.	1145.3	916
Corn [1]	medium	(125.7 g/kg bw/d ÷ 0.16 g seed weight) × 1.25 mg a.i.	982.03	785
Corn [1]	large	(69.0 g/kg bw/d ÷ 0.16 g seed weight) × 1.25 mg a.i.	539.06	431
Canola [2]	small	(146.6 g/kg bw/d ÷ 0.004 g seed weight) × 0.016 mg a.i.	586.4	36 650
Canola [2]	medium	(125.7 g/kg bw/d ÷ 0.004 g seed weight) × 0.016 mg a.i.	502.8	31 425
Canola [2]	large	(69.0 g/kg bw/d ÷ 0.004 g seed weight) × 0.016 mg a.i.	276	17 250

[1] Corn seed is treated at a rate of 1.25 mg a.i./kernel (seed weight 160 mg; 7.81 g/kg seed)

[2] Canola seed is treated at a rate of 16 µg a.i./seed (seed weight 4 mg each; 4 g a.i./kg seed)

For corn, the LD$_{50}$ for individual mouse (body weight 33 g) will be 12.83 mg a.i./individual. This dose would be acquired by ingesting 10.26 seeds (equivalent to 310.9 seeds/kg bw). Based on the number of seeds that would be ingested by the wild mammal to reach the mouse LD$_{50}$ level, the calculated margins of safety to small, medium and large wild mammals are 0.34, 0.4 and 0.72, respectively. Clothianidin, therefore, poses a moderate risk to wild mammals on an acute basis when used as a seed treatment for corn.

For canola, this LD$_{50}$ would be acquired by ingesting 802 seeds (equivalent to 24 303 seeds/kg bw). Based on the number of seeds that would be ingested by the wild mammal to reach the mouse LD$_{50}$ level, the calculated margins of safety to small, medium and large wild mammals are 0.66, 0.77 and 1.4, respectively. Clothianidin, therefore, poses a moderate risk to small and medium sized wild mammals, but no appreciable risk to large sized wild mammals on an acute basis when used as a seed treatment for canola.

It should be noted that canola seed is too small for consumption by medium mammals and may not pose an acute risk to these mammals. However, treated canola seed will pose an acute risk to small mammals.

Table 6.2.2.3.1 Acute risk from corn or canola seed treated with Poncho 600

Seed	Mammal size	Calculation	Dose acquired (mg a.i./kg bw/d)	Consumption required (seeds)
Corn [1]	small	(146.6 g/kg bw/d ÷ 0.16 g seed weight) × 1.25 mg a.i.	1145.3	916
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Corn [1]	large	(69.0 g/kg bw/d ÷ 0.16 g seed weight) × 1.25 mg a.i.	539.06	431
Canola [2]	small	(146.6 g/kg bw/d ÷ 0.004 g seed weight) × 0.016 mg a.i.	586.4	36 650
Canola [2]	medium	(125.7 g/kg bw/d ÷ 0.004 g seed weight) × 0.016 mg a.i.	502.8	31 425
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It should be noted that canola seed is too small for consumption by medium mammals and may not pose an acute risk to these mammals. However, treated canola seed will pose an acute risk to small mammals.

6.3 Risk mitigation

As discussed in sections 5.7 and 6.2.1, clothianidin, when used as seed treatment, will pose a risk to the environment. A precautionary statement is required on the product label.

Clothianidin will also pose a moderate to high risk to wild birds on an acute, dietary and reproductive basis, and a moderate risk to wild mammals on an acute toxicity basis. The risk to wild birds and wild mammals can be mitigated by precautionary label statements requiring clean-up of spilled seed at the end rows in the field. Clothianidin may pose a risk to honey bees and other pollinators, if exposure occurs via pollen and nectar of crop plants grown from treated seeds.

Mitigative measures
This chemical demonstrates the properties and characteristics associated with chemicals detected in ground water. The use of Poncho 600 in areas where soil is permeable, particularly where water table is shallow, may result in ground water contamination.

This product is toxic to birds and wild mammals when used as a seed treatment. Do not leave treated seeds on the soil surface. Any spilled or exposed seeds should be incorporated into the soil or otherwise cleaned-up from the soil surface.

7.0 Efficacy

7.1 Effectiveness

7.1.1 Intended uses

The end-use product, Poncho 600, is a flowable suspension formulation that contains 48% by weight of clothianidin (600 g/L). This is used for seed treatment to control several insect pests on canola/rapeseed and corn. The proposed use-site category for this product is USC 10—Seed Treatments Food and Feed. The proposed use claims and associated application rates are summarised in the following table.

Proposed use claims			
Crop site	**Insect pests**	**Application rate**	**Remarks**
Canola Rapeseed	Flea beetle	150 g a.i./100 kg seed	For use under low to moderate pest pressure.
		200 g a.i./100 kg seed	For use under moderate pest pressure.
		400 g a.i./100 kg seed	For use under high to extreme pest pressure where extended control is required.
		600 g a.i./100 kg seed	For use in high-value and specialty oil canola under high to extreme pest pressure where extended control is required.
Corn (including field, sweet and pop)	Corn rootworm	1.25 mg a.i./kernel	
	Corn flea beetle, black cutworm, seed corn maggot, wireworm, white grub (European chafer, June beetle and Japanese beetle)	0.25–0.5 mg a.i./ kernel	

7.1.2 Mode of action

Clothianidin is a broad-spectrum insecticide that belongs to the chemical compound class of neonicotinoids. Neonicotinoids are believed to interfere with the nicotinic acetylcholine receptors of the insect's nervous system, although different compounds may have specific binding site(s) or receptor(s). Clothianidin has a different mode of action than organophosphate, carbamate and pyrethroid insecticides. Clothianidin is reported to display systemic activity and act through contact and ingestion.

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		600 g a.i./100 kg seed	For use in high-value and specialty oil canola under high to extreme pest pressure where extended control is required.
Corn (including field, sweet and pop)	Corn rootworm	1.25 mg a.i./kernel	
	Corn flea beetle, black cutworm, seed corn maggot, wireworm, white grub (European chafer, June beetle and Japanese beetle)	0.25–0.5 mg a.i./ kernel	

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7.1.3 Crops

Crop sites are presented in Section 7.1.1.

7.1.4 Effectiveness against pests

Canola and rapeseed

Flea beetles
Fifty-one research trials were carried out in various parts of Canada between 1998 and 2001 to test the effectiveness of Poncho 600 in controlling flea beetle (*Phyllotreta sp.*) on canola. Performance was measured by foliar damage and yield. The submitted data showed that clothianidin could provide effective control of flea beetle on canola and rapeseed at various application rates, under different pest pressures and for various length of time. These rates are presented below, expressed in terms of grams of the active ingredient per 100 kg of seed. The proposed four application rates were assessed based on the submitted efficacy data.

150 g and 200 g rates: The two rates were proposed to be used under low to moderate pest pressures. The trials conducted for this submission tested various application rates (100 g, 200 g, 300 g, 400 g, 600 g and 800 g), but not the proposed 150 g rate. For the 150 g application rate, the applicant obtained authorisation from Gustafson, maker of Titan FL, to use efficacy data for Titan FL in support of its use claims. Like Poncho 600, Titan FL is a flowable suspension formulation containing clothianidin; however, it has a lower clothianidin guarantee (120 g vs. 600 g per litre) and contains two fungicide active ingredients. With the trials, the applicant requested that efficacy data for Titan FL be used. It is considered appropriate to extrapolate Titan FL data to support this application rate for Poncho 600.

PMRA's full efficacy review on Titan FL determined that 150 g a.i./100 kg seed is the lowest effective application rate for control of flea beetle on canola and rapeseed and provides adequate control of flea beetle at early plant growth stage under low to moderate pest pressures. No significant, measurable difference was found between 150 g rate and 200 g rate. Therefore, the proposed 200 g rate is not supported.

400 g and 600 g rates: These two rates were proposed for moderate-to-high or extreme pest pressures. Though not proposed, an 800 g rate was considered. Of the 51 trials submitted, 14 trials either had very low pest pressure (the commercially acceptable damage threshold is 25% foliar damage) or did not assess the foliar damage. The exclusion by the applicant of these trials in the Efficacy Summary Table is considered appropriate. As a result, only data from the remaining 37 trials are included in the assessment.

In 12 of these trials, the proposed application rates, 400 g and 600 g, were assessed along side other rates (100 g, 300 g and 800 g). In the other 25 trials, the 600 g rate was not assessed, but both lower and higher rates (including 400 g and 800 g) were tested. No significant difference in foliar damage control is found between 400 g and 600 g rates at any of the growth stages assessed, from cotyledon to 6-leaf stage. No significant difference in yield was found between the two rates. The 400 g and 600 g rates respectively provided 172% and 168% yield, compared to the untreated control. A small but significant difference in foliage damage control is found between 400 g and 800 g through meta-analysis of 37 trial-data points (mean control % values of 83.6% vs. 88.7%, mean damage level of 9.2% vs. 6.5%). The fact that a barely appreciable difference occurred only when using 800 g rate further confirms that the 600 g rate is very unlikely to provide better foliar protection than the 400 g rate. Therefore, the proposed 400 g rate is supported and 600 g rate is not. Although the applicant did not request the 800 g rate, the value of using this high rate is questionable, given that the differences between 400 g and 800 g rate are so small.

Although efficacy trials were conducted only on canola crops, the similarity between canola and rapeseed in terms of seed morphological and physiological characteristics, growth patterns, agronomy and insect/crop interaction (in regard to a same pest) suggests that a proven effective product for control of flea beetle on canola is most likely to be efficacious in controlling the same pest on rapeseed. Use patterns (e.g., application rate) of registered seed treatment products for the protection of canola and rapeseed from insect damage indicate that an equivalent control effect can be expected on both crops in regard to flea beetle control. Therefore, efficacy data to support the use of Poncho 600 to control flea beetles on canola can be accepted to support such a use on rapeseed.

To conclude, adequate efficacy data submitted support the use of Poncho 600 Seed Treatment Insecticide as a seed treatment for protection of canola and rapeseed seedlings against flea beetle damage at application rates of 150 g a.i./100 kg seed for use under low to moderate pest pressure, and 400 g a.i./100 kg seed for use under high to extreme pest pressure where extended control is required.

Corn (sweet, field and pop)

Corn rootworm

Thirty-one small plot field trials were conducted in Ontario (4) and the United States (27) from 1997 to 2000 to assess the effectiveness of Poncho 600 in controlling corn rootworm, including western corn rootworm (*Diabrotica v. virgifera*) and northern corn rootworm (*Diabrotica barberi*). A number of different rates were tested (0.25 to 2.70 mg a.i./kernel) and performance was compared to an untreated control and commercial standards. Performance was measured by root damage ratings, lodging counts, emergence counts and yield. The submitted data showed that Poncho 600, applied at rates ranging from 0.25 to 2.7 mg a.i./kernel, reduced feeding damage by corn rootworm, when compared with the untreated control. Root damage ratings from plots treated with Poncho 600 applied at 1.25 to 2.7 mg a.i./kernel were almost half those observed in the untreated control, and similar to those from plots treated with the commercial standards (Force 3.0G and Counter 20G). No rate response was observed above 1.25 mg a.i./kernel. Therefore, the efficacy data fully supported the proposed application rate of 1.25 mg a.i. per kernel for control of corn rootworm.

Corn flea beetle

Twenty small plot field trials were conducted in Ontario (1) and the United States (19) from 1997 to 2000 to assess the effectiveness of Poncho 600 in controlling corn flea beetle (*Chaetocnema pulicaria*) and the incidence of Stewart's Wilt (*Erwinia stewartii*) a bacterium that is vectored by corn flea beetles. A number of different rates were tested (0.13 to 2.5 mg a.i./kernel) and performance was compared to an untreated control and commercial standards (Gaucho FS). Performance was measured mainly by incidence of diseased plants. Only three of the corn flea beetle trials measured yields and two of these trials tested only one rate, which was above the proposed label rate.

Both proposed application rates (0.25 mg and 0.5 mg) provided a certain degree of control against corn flea beetle, in terms of reduction in Stewart's Wilt disease incidence. However, a statistically significant difference in control (about 10%) was found between 0.25 mg and 0.5 mg rates, based on the data from eight side-by-side trials comparing the two rates. The rate of 0.5 mg provided 68% control while 0.25 mg gave 57%. Therefore, the proposed rate range of 0.25 to 0.5 mg a.i. per kernel is justified to accommodate various control needs.

Black cutworm

Fourteen small plot field trials were conducted in Ontario (2) and the United States (12) from 1997 to 2000 to assess the effectiveness of Poncho 600 in controlling black cutworm, Agrostis ipsilon). A number of different rates were tested (0.13 to 2.50 mg a.i./kernel) and performance was compared to an untreated or fungicide-treated control and commercial standards (Lorsban 15G, Lorsban 4E, Force 1.5G, Pounce 384 EC and Ambush 500 EC). For the majority of the trials, performance was measured by assessment of the damage (number of cut or injured plants). Yield was only recorded in plots where Poncho 600 was applied at 1.0 to 2.5 mg a.i. per kernel, which is not within the proposed rate range for Poncho 600.

Poncho 600 provided good control, as measured by reduction in number of cut plants, ranging from 55 to 96% at rates of 0.13 to 2.50 mg a.i./kernel. There appeared to be a rate response to clothianidin from 0.13 to 0.5 mg a.i./kernel. On average, 0.5 mg seemed to perform better than 0.25 mg (57% vs. 43%, based on the parameter of "injured plant"). However, no statistically significant difference in damage control against black cutworm is found between 0.25 mg and 0.5 mg based on the data from four side-by-side trials comparing 0.25 and 0.5 mg rates. It is possible that the apparent rate response observed in trials against black cutworm could become significant if there had been more data points. Based on the overall assessment of product use patterns against other insect pests on corn (e.g., 1.25 mg for control of rootworm and 0.25 to 0.5 mg for control of flea beetle), the proposed rate range of 0.25 mg to 0.5 mg a.i. per kernel is considered acceptable for control of black cutworm.

Wireworm

Eleven small plot field trials were conducted in Ontario (3) and the United States (8) from 1998 to 2000 to assess the effectiveness of Poncho 600 in controlling wireworms (Family *Elateridae*). A number of different rates were tested (0.125 to 1.35 mg a.i./kernel) and performance was compared to an untreated or fungicide-treated control and commercial standards (Lorsban 15G, Force 3.0G). Performance (control %) was determined from the number of dead, damaged and stunted plants per treatment relative to the untreated.

Poncho 600 provided good control, as measured by reduction in damaged plants, from 64 to 73% at various rates. No statistically significant difference in damage control against wireworm is found between 0.25 mg and 0.5 mg based on the data from four side-by-side trials comparing 0.25 and 0.5 mg rates (67% vs. 73% control). A lowest effective rate could not be determined. However, based on the overall assessment of product use patterns against various other insect pests on corn (e.g., 1.25 mg for control of rootworm and 0.25–0.5 mg for control of flea beetle), the proposed rate range from 0.25 mg to 0.5 mg a.i. per kernel is considered acceptable for control of wireworm.

Seed corn maggot

Two small plot field trials were conducted in 2000, one in Kentucky and one in Ohio, to assess the effectiveness of Poncho 600 in controlling seed corn maggot (*Delia platura*). Performance of Poncho 600 was tested at 0.125, 0.25 and 0.5 mg a.i./kernel and compared to an untreated control as well as the commercial standards (Gaucho 480 FS and Force ST). Performance was measured by emergence counts and plant stand counts.

Control of seed corn maggot, as measured by plant stand, was 25 to 30% higher in plots treated with Poncho 600 than in the control plots, and was greater than in the plots treated with Gaucho 600 and Force ST. A rate response was observed in plant emergence counts, but not in plant stand counts. Due to limited numbers of trials, a lowest effective rate could not be determined. However, based on the overall assessment of product performance against various other insect pests on corn (e.g., 1.25 mg for control of rootworm and 0.25–0.5 mg for control of flea beetle), the proposed rate range from 0.25 to 0.5 mg a.i. per kernel is considered acceptable for control of seed corn maggot.

White grubs

Data from seventeen trials were submitted to support use claims for control of European chafer (6 trials on corn and 4 trials on soybean in Ontario), June beetle (3 trials on corn in the United States) and Japanese beetle (4 trials on corn in the United States). Product performance was assessed in terms of plant stand counts, plant vigour or damaged plant counts.

The data showed that Poncho 600 applied at 0.25 mg a.i./kernel was as effective as the same product applied at higher rates (up to 1.25 mg a.i./kernel) and equal to or better than commercial standards (e.g., Gaucho) in controlling these three pests. Although the Japanese beetle and the June beetle still remain as turf pests, the potential of "host expanding" as observed in European chafer cannot be excluded. Due to the similarities of damage patterns of the three species (they attack plants at similar sites and only the larval stage of these species causes the damage), a general term of control claims for "white grubs" that includes common names for all these species (European chafer, Japanese beetle and June beetle) in brackets can be acceptable for the Poncho 600 label.

To conclude, an effective application rate has been reliably established at 0.25 mg (a.i. per kernel) for control of white grubs on corn.

7.2 Phytotoxicity to target plants (including different cultivars), or to target plant products

Canola and rapeseed

Phytotoxicity was evaluated in thirty-six small plot field trials. Phytotoxic effect was assessed using stand counts (12 trials), yield (9 trials) and foliar symptoms of phytotoxicity (34 trials). Symptoms of phytotoxicity included a halo-like effect (e.g., a narrow band of yellow or white) along the margins of cotyledons, occasionally accompanied by a slight cupping of the leaf margin. Phytotoxicity was calculated as the percentage of plants in a 50- or 100-plant subsample per plot (usually taken from one or two rows) displaying symptoms on at least one cotyledon. Evaluations were carried out at the cotyledon, 2-leaf, and 3- to 6-leaf growth stages. Germination was also evaluated in seventeen laboratory trials, ten trials after short-term storage (1 to 6 months) and seven after long-term storage (6.5 to 16.5 months). Trials were carried out on various varieties of canola and mustard. Seed was stored at room temperature in an outside shed from receipt following field trials until germination testing. A subsample was taken from relevant treatments and sent to Discovery Seeds (Saskatoon, Saskatchewan) for germination tests.

Symptoms of phytotoxicity were observed in all years on the canola cotyledons. However, the symptoms were transient and generally disappeared within two weeks. In general, much less than 50% of cotyledon surface area had phytotoxic symptoms ("50%" is a standard set by the Canadian Food Inspection Agency for "abnormal" seed testing for *Brassica*). Moreover, despite early symptoms, all treatment plots, regardless of application rates, showed similar stand counts at both the cotyledon and 2- to 5-leaf stages and similar yields.

Germination of canola seed exceeded 90% in all laboratory trials following short-term storage (1 to 6 months). Furthermore, there was no significant difference in germination of untreated seed and seed treated with Poncho 600 at any of the proposed rates, except in one trial at the 400 g rate. In this trial, stand count was significantly lower than in the untreated control, but stand counts at the 800 g rate were not significantly different. This suggests that some other factor besides the 400 g rate may have caused the low germination.

To conclude, there is no significant phytotoxicity as a result of seed treatment of canola and rapeseed.

Corn

Eighty-one small plot field trials and two laboratory research trials were conducted to assess the phytotoxicity of Poncho 600 on corn. Nineteen of the small plot field trials were carried out on sweet corn and sixty-two on field corn. In the field trials, performance was assessed by counts of emergence and yield, and by visual assessments of crop injury. The laboratory trials were carried out on a number of different corn varieties, using both standard laboratory paper towel seed germination testing at 25°C, standard Iowa Cold Stess germination testing (cold stress at 10°C followed by germination test 25°C) and standard greenhouse sterile sand/soil testing. Assessment of performance included counts of germination and abnormal seeds.

The data presented showed that clothianidin does not cause any significant phytotoxic damage to corn seeds and plants at the proposed application rates. However, the data did show that long-term storage could have negative effects, and thereby the label caution for carry-over of treated seed is accepted.

7.3 Observations on undesirable or unintended side effects

7.3.1 Impact on succeeding crops

Not applicable.

7.3.2 Impact on adjacent crops

Not applicable.

7.4 Economics

Not assessed.

7.5 Sustainability

7.5.1 Survey of alternatives

The major alternative insecticide active ingredients currently registered for seed treatment to control the pests on the proposed labels of Poncho 600 include, but are not necessarily limited to, the following.

Pest	Available alternative active ingredients for seed treatment	Remarks
Flea beetle (canola and rapeseed)	neonicotinoids (acetamiprid, imidacloprid, thiamethoxam)	
Corn rootworm (corn)	none	
Corn flea beetle (corn)	imidacloprid	
Corn wireworm (corn)	imidacloprid	
Seed corn maggot (corn)	diazinon	
White grubs (corn)	none	White grubs are new pests for corn in Canada.

7.5.2 Contribution to risk reduction

Clothianidin is potentially an alternative to organophosphate insecticides for control of the pests on the proposed label of Poncho 600. With a similar mode of action to other chloro-nicotinic compounds, clothianidin is an alternative to lindane and diazinon seed treatment products for the proposed seed treatment uses on the label of Poncho 600. Both organophosphate and lindane insecticides are currently undergoing re-evaluation by the PMRA and the USEPA.

7.5.3 Information on the occurrence or possible occurrence of the development of resistance

Clothianidin is a broad-spectrum insecticide that belongs to the chemical compound class of neonicotinoids. Neonicotinoids are believed to interfere with the nicotinic acetylcholine receptors of the insect's nervous system, although different compounds may have specific binding site(s) or receptor(s). Other neonicotinoid insecticides currently registered in Canada include acetamiprid, imidacloprid and thiamethoxam. According to

7.5 Sustainability

7.5.1 Survey of alternatives

The major alternative insecticide active ingredients currently registered for seed treatment to control the pests on the proposed labels of Poncho 600 include, but are not necessarily limited to, the following.

Pest	Available alternative active ingredients for seed treatment	Remarks
Flea beetle (canola and rapeseed)	neonicotinoids (acetamiprid, imidacloprid, thiamethoxam)	
Corn rootworm (corn)	none	
Corn flea beetle (corn)	imidacloprid	
Corn wireworm (corn)	imidacloprid	
Seed corn maggot (corn)	diazinon	
White grubs (corn)	none	White grubs are new pests for corn in Canada.

7.5.2 Contribution to risk reduction

Clothianidin is potentially an alternative to organophosphate insecticides for control of the pests on the proposed label of Poncho 600. With a similar mode of action to other chloro-nicotinic compounds, clothianidin is an alternative to lindane and diazinon seed treatment products for the proposed seed treatment uses on the label of Poncho 600. Both organophosphate and lindane insecticides are currently undergoing re-evaluation by the PMRA and the USEPA.

7.5.3 Information on the occurrence or possible occurrence of the development of resistance

Clothianidin is a broad-spectrum insecticide that belongs to the chemical compound class of neonicotinoids. Neonicotinoids are believed to interfere with the nicotinic acetylcholine receptors of the insect's nervous system, although different compounds may have specific binding site(s) or receptor(s). Other neonicotinoid insecticides currently registered in Canada include acetamiprid, imidacloprid and thiamethoxam. According to

Regulatory Directive DIR99-06, *Voluntary Pesticide Resistance-Management Labelling Based on Target Site/Mode of Action*, the following statements should be incorporated on the label of Poncho 600 Seed Treatment Insecticide.

GROUP	**4**	**INSECTICIDE**

Resistance management recommendations
For resistance management, Poncho 600 contains a Group 4 insecticide. Any insect population may contain individuals naturally resistant to Poncho 600 and other Group 4 insecticides. The resistant biotypes may dominate the insect population if these insecticides are used repeatedly in the same field. Other resistance mechanisms that are not linked to site of action, but specific for individual chemicals, such as enhanced metabolism, may also exist. Appropriate resistance management strategies should be followed.

To delay insecticide resistance:

- Where possible, rotate the use of Poncho 600 or other Group 4 insecticides with different groups that control the same pests in a field.
- Insecticide use should be based on an integrated pest management (IPM) program that includes scouting and record keeping and considers cultural, biological and other chemical control practices.
- Monitor treated pest populations for resistance development.
- Contact your local extension specialist or certified crop advisors for any additional pesticide resistance management and IPM recommendations for the specific site and pest problems in the area.
- For further information or to report suspected resistance, contact Bayer CropScience at 1-888-283-6847.

7.6 Conclusions

Adequate efficacy and value data fully support the amended label claims for seed treatment at the approved application rates (see Appendix VI, Value summary) for control of flea beetle on canola and rapeseed, and for control of corn rootworm, corn flea beetle, black cutworm, seed corn maggot, wireworm and white grubs on corn.

8.0 Toxic Substances Management Policy

During the review of clothianidin insecticide and the end-use product Poncho 600, the PMRA has taken into account the federal Toxic Substances Management Policy (TSMP)[1] and has followed PMRA Regulatory Directive DIR99-03[2]. It has been determined that this active ingredient and its end-use products do not meet TSMP Track 1 criteria for the following reasons.

Clothianidin meets the criterion for persistence in soil and water. Its value for half-life in soil (495–990 d) and water (1732 d) are above the TSMP Track 1 cut-off criteria for soil and water (≥ 182 d). Clothianidin is unlikely to volatilize, based on the vapour pressure and Henry's Law constant. Therefore, a study of persistence in air was not triggered. The half-life of clothianidin in sediment is 37 d, which is below the TSMP cutoff criterion for sediment (≥ 365 days).

Clothianidin is not bioaccumulative. The log K_{ow} was 0.7, which is below the TSMP Track 1 cut-off criterion of log $K_{ow} \geq 5$.

The toxicity of clothianidin is described in sections 3.0 and 6.0.

Clothianidin does not form any major transformation products that meet the TSMP Track 1 criteria.

Technical grade clothianidin does not contain any by-products or microcontaminants that meet the TSMP Track 1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials nor are they expected to be generated during the manufacturing process.

The formulated product (Poncho 600) does not contain any formulants, by-products or microcontaminants that are known to be TSMP Track 1 substances. In addition, all formulants are classified as USEPA List 3 or List 4B formulants.

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[1] The federal Toxic Substances Management Policy is available through Environment Canada's website at www.ec.gc.ca/toxics

[2] *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*, DIR99-03, is available through the Pest Management Information Service. Phone 1-800-267-6315 within Canada or 1-613-736-3799 outside Canada (long distance charges apply); Fax: (613) 736-3798.

9.0 Regulatory decision

9.1 Regulatory decision

Technical grade clothianidin and the associated end-use product, Poncho 600 Seed Treatment Insecticide, for seed treatment to control flea beetle on canola/rapeseed and to control corn rootworm, corn flea beetle, black cutworm, seed corn maggot, wireworm and white grub on corn have been granted temporary registration under Section 17 of the Pest Control Products Regulations, subject to submission of the following data:

- Batch data
- Storage stability data (product chemistry)
- Genotoxicity studies
- Developmental immunotoxicity studies
- Passive dosimetry or biological monitoring study
- Field crop rotation study
- Analytical methodology for sediment
- Long-term hydrolysis study
- Leaching study
- Acute oral toxicity to bumble bees and leaf-cutter bees
- Chronic toxicity to hives of honey bees under field conditions
- Acute oral toxicity to the red-winged blackbird, house sparrow and mallard duck
- Toxicity to wild birds under field use conditions.

List of abbreviations

A/G ratio	albumin-globulin ratio
a.i.	active ingredient
ALT	alanine aminotransferase
AOEL	allowable operator exposure level
AST	aspartate aminotransferase
BM	bone marrow
BUN	blood urea nitrogen
bw	body weight
bw/d	body weight/day
bwg	body-weight gain
d	day(s)
DMSO	dimethyl sulfoxide
DT50	dissipation time 50%
EEC	expected environmental concentration
EP	end-use product
EXAMS	Exposure Analysis Modeling System
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FC	food consumption
FE	food efficiency
FOB	functional observational battery
g	gram(s)
GC	gas chromatography
GD	gestation day
GGT	gamma glutamyl transpeptidase
h	hour(s)
ha	hectare
h.c.	historical control
HDT	highest dose tested
Hgb	hemoglobin
HPLC	high-performance liquid chromatography
ICP	inductivity coupled plasma
K_d	absorption coefficient
K_{oc}	organic–carbon partition coefficient
K_{ow}	*n*-octanol–water partition coefficient
LC-MS/MS	high-performance liquid chromatography with tandem mass spectrometry
LADD	lifetime average daily dose
LD_{50}	lethal dose 50%
LC_{50}	lethal concentration 50%
LEACHM	Leaching Estimation and Chemistry Model
LER	lowest effective rate
LOAEL	lowest observed adverse effect level
LOQ	limit of quantitation

MAS	maximum average score
MCV	mean cell volume
nm	nanometer(s)
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
PCV	packed cell volume
PBI	plantback interval
PHI	preharvest interval
PMRA	Pest Management Regulatory Agency
PND	post-natal day
PRZM	Pesticide Root Zone Model
RBC	red blood cell
RSD	relative standard deviation
S9	exogenous metabolic activation system
SF	safety factor
STMR	supervised trial median residue
TEA	thermal energy analyzer
TTR	total radioactive residue
UF	uncertainty factor
U.S.	United States
USC	use site category
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drugs Administration
UV	ultraviolet
WBC	white blood cell
wt	weight

Appendix I Method of residue analysis

Table 1 Analytical methods for analysis of the active substance as manufactured (OECD 2.2.1)

For the product manufactured using basic Takeda process.

Product	Analyte	Method ID	Method type	Linearity range µg/mL	Mean recovery	RSD (%)	Method
Clothianidin	Active (TI-435)	6155-119	HPLC/UV at 265 nm	53.7–202	100.4	1.3	Acceptable
Clothianidin	Major impurities	6155-119	HPLC/UV at 265 nm	1–10	90.7–111.8	2.2–37.5	Acceptable

Table 2 Analytical methods for formulation analysis (OECD 2.2.2)

Product	Method ID	Method	Recovery range (%)	RSD (%)	Method
Poncho 600	C-64.01-00	HPLC/UV at 280 nm	98.9	0.209	Acceptable

Table 3 Analytical methods for residue analysis (OECD 2.2.3)

Summary of method validation data for residues of clothianidin and transformation products in soil, water and plant matrices								
Matrix	**Spiked level**	**Overall mean % recovery (n)**					**LOQ**	**Method**
		Parent TI-435	**TZNG**	**TZMU**	**MNG**	**TMG**		
Soil	5–50 ppb	100.4 (10)	97.2 (10)	96.6 (10)	101.5 (10)	96.4 (10)	5.0 ppb	Accepted
	Overall RSD (%)	2.11	2.19	3.4	1.49	1.49		
Sediment	No method provided for sediment							Not accepted
Water	0.05–0.5 ppb	86.0 (10)	*NP*	*NP*	*NP*	*NP*	0.05[1] or 10.0[2] ppb	Accepted
	Overall RSD (%)	4.6	0.1	*NP*	*NP*	*NP*		
Plant (sugar beet)	0.02–0.2 ppm	89.5 (10)	*NP*	*NP*	*NP*	*NP*	0.02 ppm	Accepted
	RSD (%)	6.3	*NP*	*NP*	*NP*	*NP*		

NP not provided

[1] For TI-435

[2] For TZNG

Appendix I Method of residue analysis

Table 1 Analytical methods for analysis of the active substance as manufactured (OECD 2.2.1)

For the product manufactured using basic Takeda process.

Product	Analyte	Method ID	Method type	Linearity range µg/mL	Mean recovery	RSD (%)	Method
Clothianidin	Active (TI-435)	6155-119	HPLC/UV at 265 nm	53.7–202	100.4	1.3	Acceptable
Clothianidin	Major impurities	6155-119	HPLC/UV at 265 nm	1–10	90.7–111.8	2.2–37.5	Acceptable

Table 2 Analytical methods for formulation analysis (OECD 2.2.2)

Product	Method ID	Method	Recovery range (%)	RSD (%)	Method
Poncho 600	C-64.01-00	HPLC/UV at 280 nm	98.9	0.209	Acceptable

Table 3 Analytical methods for residue analysis (OECD 2.2.3)

Summary of method validation data for residues of clothianidin and transformation products in soil, water and plant matrices								
Matrix	**Spiked level**	**Overall mean % recovery (n)**					**LOQ**	**Method**
		Parent TI-435	**TZNG**	**TZMU**	**MNG**	**TMG**		
Soil	5–50 ppb	100.4 (10)	97.2 (10)	96.6 (10)	101.5 (10)	96.4 (10)	5.0 ppb	Accepted
	Overall RSD (%)	2.11	2.19	3.4	1.49	1.49		
Sediment	No method provided for sediment							Not accepted
Water	0.05–0.5 ppb	86.0 (10)	*NP*	*NP*	*NP*	*NP*	0.05[1] or 10.0[2] ppb	Accepted
	Overall RSD (%)	4.6	0.1	*NP*	*NP*	*NP*		
Plant (sugar beet)	0.02–0.2 ppm	89.5 (10)	*NP*	*NP*	*NP*	*NP*	0.02 ppm	Accepted
	RSD (%)	6.3	*NP*	*NP*	*NP*	*NP*		

NP not provided

[1] For TI-435

[2] For TZNG

Appendix II Occupational exposure summary tables

Table 1 Unit exposures for seed treatment workers (Oftanol study)

Activity	Average kg a.i. handled/d	Unit exposures (μg a.i./kg a.i. handled)[a]					
		Dermal		Inhalation		Total	
		single layer + gloves	+ coveralls	no respirator	with respirator	single layer + gloves	+ coveralls + respirator
mixer/loader	67.9	187.8	177.33	1.49	0.15	189.28	178.48
coater	67.9	32.33	25.84	0.96	0.1	33.29	25.93
bagger	67.9	20.43	8.2	0.11	0.01	20.54	8.21
shift foreman	67.9	97.52	38.65	0.5	0.05	98.02	38.7

[a] 75% protection factor for cotton coveralls; 90% protection factor for respirator

Table 2 Unit exposure to workers (Baytan 312 FS study)

Activity	Average kg a.i. handled/8 h	Unit exposure (μg a.i./kg a.i. handled)[a]					
		Dermal		Inhalation		Total	
		single layer + gloves	+ coveralls	no respirator	with respirator	single layer + gloves	+ coveralls + respirator
Large facility							
mix/calibrate	24.2	375.4	99.66	0.16	0.02	375.57	99.67
treat/bag	28.1	262.66	112.7	14.14	1.41	276.79	114.11
stack/tag	12.6	129.32	42.88	4.68	0.47	133.99	43.35
forklift operator	36	13.35	6	1.09	0.11	14.44	6.11
Medium facility							
mix/calibrate	12.1	7411.46	1872.5	0.09	0.01	7411.55	1872.5
bag	18.8	82.66	47.76	56.4	5.64	139.06	53.4
tag/sew	19.9	107.83	59.64	135.15	13.52	242.98	73.16
stack	9.8	104.5	44.75	21.03	2.1	125.53	46.86

Activity	**Average kg a.i. handled/8 h**	**Unit exposure (µg a.i./kg a.i. handled)** [a]					
		Dermal		**Inhalation**		**Total**	
		single layer + gloves	**+ coveralls**	**no respirator**	**with respirator**	**single layer + gloves**	**+ coveralls + respirator**
Small facility							
mix/calibrate	12.1	226.19	98.21	0.08	0.01	226.27	98.22
treat/bag/ sew/stack	1.8	682.13	277.36	230.84	23.08	912.97	300.44

[a] 75% protection factor for cotton coveralls; 90% protection factor for respirator

Table 3 Total unit exposures and systemic exposures for seed treatment workers [a]

Activity (facility)	**Total unit exposure** [b] **(µg a.i./kg a.i.)**		**Seed**	**kg a.i. handled/d**	**Systemic exposure** [b] **(mg/kg bw/d)**	
	50%	**1%**			**50%**	**1%**
mix/load/calibrate + coat (typical facility)	232	4.81	canola	160	0.53	0.01
			corn	246	0.82	0.02
all tasks (mix/load/calibrate/treat/bag/stack) (small facility)	381	30.3	canola	80	0.44	0.03
			corn	82	0.45	0.04
bag/tag/sew/stack (typical facility)	29.2	4.04	canola	160	0.07	0.01
			corn	246	0.1	0.02
mix/load/calibrate (typical facility) (90th percentile)	578	11.7	canola	160	1.32	0.03
			corn	246	2.03	0.04
all tasks (mix/load/calibrate/treat/bag/stack) (small facility) (90th percentile)	795	69.6	canola	80	0.91	0.08
			corn	82	0.93	0.08

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator and chemical-resistant gloves

[b] Based on 50% or 1% dermal absorption

Appendix II Occupational exposure summary tables

Table 1 Unit exposures for seed treatment workers (Oftanol study)

Activity	**Average kg a.i. handled/d**	**Unit exposures (µg a.i./kg a.i. handled) [a]**					
		Dermal		**Inhalation**		**Total**	
		single layer + gloves	**+ coveralls**	**no respirator**	**with respirator**	**single layer + gloves**	**+ coveralls + respirator**
mixer/loader	67.9	187.8	177.33	1.49	0.15	189.28	178.48
coater	67.9	32.33	25.84	0.96	0.1	33.29	25.93
bagger	67.9	20.43	8.2	0.11	0.01	20.54	8.21
shift foreman	67.9	97.52	38.65	0.5	0.05	98.02	38.7

[a] 75% protection factor for cotton coveralls; 90% protection factor for respirator

Table 2 Unit exposure to workers (Baytan 312 FS study)

Activity	**Average kg a.i. handled/8 h**	**Unit exposure (µg a.i./kg a.i. handled) [a]**					
		Dermal		**Inhalation**		**Total**	
		single layer + gloves	**+ coveralls**	**no respirator**	**with respirator**	**single layer + gloves**	**+ coveralls + respirator**
Large facility							
mix/calibrate	24.2	375.4	99.66	0.16	0.02	375.57	99.67
treat/bag	28.1	262.66	112.7	14.14	1.41	276.79	114.11
stack/tag	12.6	129.32	42.88	4.68	0.47	133.99	43.35
forklift operator	36	13.35	6	1.09	0.11	14.44	6.11
Medium facility							
mix/calibrate	12.1	7411.46	1872.5	0.09	0.01	7411.55	1872.5
bag	18.8	82.66	47.76	56.4	5.64	139.06	53.4
tag/sew	19.9	107.83	59.64	135.15	13.52	242.98	73.16
stack	9.8	104.5	44.75	21.03	2.1	125.53	46.86

Activity	Average kg a.i. handled/8 h	Unit exposure (μg a.i./kg a.i. handled)[a]					
		Dermal		Inhalation		Total	
		single layer + gloves	+ coveralls	no respirator	with respirator	single layer + gloves	+ coveralls + respirator
Small facility							
mix/calibrate	12.1	226.19	98.21	0.08	0.01	226.27	98.22
treat/bag/ sew/stack	1.8	682.13	277.36	230.84	23.08	912.97	300.44

[a] 75% protection factor for cotton coveralls; 90% protection factor for respirator

Table 3 Total unit exposures and systemic exposures for seed treatment workers[a]

Activity (facility)	Total unit exposure[b] (μg a.i./kg a.i.)		Seed	kg a.i. handled/d	Systemic exposure[b] (mg/kg bw/d)	
	50%	1%			50%	1%
mix/load/calibrate + coat (typical facility)	232	4.81	canola	160	0.53	0.01
			corn	246	0.82	0.02
all tasks (mix/load/calibrate/treat/ bag/stack) (small facility)	381	30.3	canola	80	0.44	0.03
			corn	82	0.45	0.04
bag/tag/sew/stack (typical facility)	29.2	4.04	canola	160	0.07	0.01
			corn	246	0.1	0.02
mix/load/calibrate (typical facility) (90th percentile)	578	11.7	canola	160	1.32	0.03
			corn	246	2.03	0.04
all tasks (mix/load/calibrate/treat/ bag/stack) (small facility) (90th percentile)	795	69.6	canola	80	0.91	0.08
			corn	82	0.93	0.08

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator and chemical-resistant gloves

[b] Based on 50% or 1% dermal absorption

Table 4 Margins of exposure for seed treatment workers [a]

Activity (facility)	Seed	MOE [b]	
		50%	1%
Intermediate exposure duration [c]			
mix/load/calibrate + coat (typical facility)	canola	18	890
	corn	12	580
all tasks (small facility) (mix/load/calibrate/treat/bag/stack)	canola	23	280
	corn	22	280
bag/tag/sew/stack (typical facility)	canola	147	1060
	corn	96	690
Acute exposure duration [d]			
mix/load/calibrate + coat (typical facility) (90th percentile)	canola	19	940
	corn	12	610
all tasks (small facility) (mix/load/calibrate/treat/bag/stack) (90th percentile)	canola	27	310
	corn	27	310

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator, chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

[c] Intermediate-term NOAEL = 9.8 mg/kg bw/d; target MOE = 300

[d] Acute-term NOAEL = 25 mg/kg bw/d; target MOE = 100

Table 5 Lifetime average daily doses for seed treatment workers [a]

Activity (facility)	Canola		Corn	
	LADD (mg/kg bw/d) [b]		LADD (mg/kg bw/d) [b]	
	50%	1%	50%	1%
mix/load/calibrate + coat (typical facility)	0.07	0.001	0.11	0.002
all tasks (mix/load/calibrate/treat/bag/stack) (small facility)	0.06	0.005	0.06	0.005
bag/tag/sew/stack (typical facility)	0.009	0.001	0.01	0.002

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Table 6 Cancer risk levels for seed treatment workers [a]

Activity (facility)	Canola		Corn	
	Risk level [b]		Risk level [b]	
	50%	1%	50%	1%
mix/load/calibrate + coat (typical facility)	1.6×10^{-4}	3.4×10^{-6}	2.5×10^{-4}	5.2×10^{-6}
all tasks (mix/load/calibrate/treat/bag/stack) (small facility)	1.3×10^{-4}	1.1×10^{-5}	1.4×10^{-4}	1.1×10^{-5}
bag/tag/sew/stack (typical facility)	2.0×10^{-5}	2.8×10^{-6}	3.1×10^{-5}	4.4×10^{-6}

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Table 7 Exposure estimates for workers planting treated seed [a]

Activity	Unit exposure [b] (mg a.i./kg a.i.)		Crop	Systemic exposure [b] (mg/kg bw/d)	
	50%	1%		50%	1%
planting	0.213	0.01	canola	0.007	0.0002
			corn	0.016	0.0004

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Default dermal absorption of 50% or 1%

Table 8 Margins of exposure for workers planting treated seed [a]

Activity	Seed	MOE [b]	
		50%	1%
***Short-term exposure duration* [c]**			
planting treated seed	canola	1400	53 000
	corn	600	24 000

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

[c] Short-term NOAEL = 9.8 mg/kg bw/d; target MOE = 300

Table 4 Margins of exposure for seed treatment workers [a]

Activity (facility)	Seed	MOE [b]	
		50%	1%
Intermediate exposure duration [c]			
mix/load/calibrate + coat (typical facility)	canola	18	890
	corn	12	580
all tasks (small facility) (mix/load/calibrate/treat/bag/stack)	canola	23	280
	corn	22	280
bag/tag/sew/stack (typical facility)	canola	147	1060
	corn	96	690
Acute exposure duration [d]			
mix/load/calibrate + coat (typical facility) (90th percentile)	canola	19	940
	corn	12	610
all tasks (small facility) (mix/load/calibrate/treat/bag/stack) (90th percentile)	canola	27	310
	corn	27	310

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator, chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

[c] Intermediate-term NOAEL = 9.8 mg/kg bw/d; target MOE = 300

[d] Acute-term NOAEL = 25 mg/kg bw/d; target MOE = 100

Table 5 Lifetime average daily doses for seed treatment workers [a]

Activity (facility)	Canola		Corn	
	LADD (mg/kg bw/d) [b]		LADD (mg/kg bw/d) [b]	
	50%	1%	50%	1%
mix/load/calibrate + coat (typical facility)	0.07	0.001	0.11	0.002
all tasks (mix/load/calibrate/treat/bag/stack) (small facility)	0.06	0.005	0.06	0.005
bag/tag/sew/stack (typical facility)	0.009	0.001	0.01	0.002

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Table 6 Cancer risk levels for seed treatment workers [a]

Activity (facility)	Canola		Corn	
	Risk level [b]		Risk level [b]	
	50%	1%	50%	1%
mix/load/calibrate + coat (typical facility)	1.6×10^{-4}	3.4×10^{-6}	2.5×10^{-4}	5.2×10^{-6}
all tasks (mix/load/calibrate/treat/bag/stack) (small facility)	1.3×10^{-4}	1.1×10^{-5}	1.4×10^{-4}	1.1×10^{-5}
bag/tag/sew/stack (typical facility)	2.0×10^{-5}	2.8×10^{-6}	3.1×10^{-5}	4.4×10^{-6}

[a] PPE of coveralls over long-sleeved shirt and long pants, respirator and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Table 7 Exposure estimates for workers planting treated seed [a]

Activity	Unit exposure [b] (mg a.i./kg a.i.)		Crop	Systemic exposure [b] (mg/kg bw/d)	
	50%	1%		50%	1%
planting	0.213	0.01	canola	0.007	0.0002
			corn	0.016	0.0004

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Default dermal absorption of 50% or 1%

Table 8 Margins of exposure for workers planting treated seed [a]

Activity	Seed	MOE [b]	
		50%	1%
Short-term exposure duration [c]			
planting treated seed	canola	1400	53 000
	corn	600	24 000

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

[c] Short-term NOAEL = 9.8 mg/kg bw/d; target MOE = 300

Table 9 Lifetime average daily doses for workers planting treated seed [a]

Activity	Canola		Corn	
	LADD (mg/kg bw/d) [b]		LADD (mg/kg bw/d) [b]	
	50%	1%	50%	1%
planting treated seed	0.0001	0	0.0002	0

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Table 10 Cancer risk levels for workers planting treated seed [a]

Activity	Canola		Corn	
	Risk level [b]		Risk level [b]	
	50%	1%	50%	1%
planting treated seed	2.5×10^{-7}	6.2×10^{-9}	5.6×10^{-7}	1.4×10^{-8}

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Table 9 Lifetime average daily doses for workers planting treated seed [a]

Activity	Canola		Corn	
	LADD (mg/kg bw/d) [b]		LADD (mg/kg bw/d) [b]	
	50%	1%	50%	1%
planting treated seed	0.0001	0	0.0002	0

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Table 10 Cancer risk levels for workers planting treated seed [a]

Activity	Canola		Corn	
	Risk level [b]		Risk level [b]	
	50%	1%	50%	1%
planting treated seed	2.5×10^{-7}	6.2×10^{-9}	5.6×10^{-7}	1.4×10^{-8}

[a] PPE of long-sleeved shirt and long pants and chemical-resistant gloves

[b] Based on default dermal absorption of 50% or 1%

Appendix III Toxicology summary table

METABOLISM: RAT

Rate and extent of absorption and excretion: TI-435 was readily absorbed and excreted within 96 hours following a single 2.5 mg/kg bw or repeated oral dose of 25 mg/kg bw. At a dose of 250 mg/kg bw, absorption became biphasic and was saturated. Urinary excretion accounted for 89.2–94.6% of the administered radioactivity. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups (3.8–8.6%).

Distribution/target organ(s): There was a rapid absorption and distribution of administered radioactivity to all organs and tissues, followed by rapid excretion with reduction to background levels in most tissues and organs within 24 hours. Gender-related differences in plasma kinetics were indicated by the somewhat greater rate of absorption and elimination in females. Since tissue burdens were very low, neither TI-435 nor its metabolites appear to undergo significant sequestration.

Toxicologically significant compound(s): The metabolites identified (primarily oxidative demethylation products and cleavage products of the nitrogen-carbon bond between the nitroimino and thiazolyl moieties) were consistent with Phase I processes. Major metabolites found in composite urine samples were TZNG (7–12.5%), MNG (7.8–13.2%) and NTG (1.4–3.9% of administered).

METABOLISM: MICE

Rate and extent of absorption and excretion: TI-435 was readily absorbed and excreted within 168 hours following a single oral dose of 5 mg/kg bw. Urine was the major route of excretion of TI-435, accounting for 92.4–93.7% of the administered radioactivity. Feces accounted for 5.0–6.8% of the administered radioactivity. Within 24 hours, 89.0–91.7% of the administered radioactivity was excreted in the urine and 4.9–6.2% was excreted in the feces.

Distribution/target organ(s): Residual radioactivity in any given tissue at 168 hours post-dose was considerably less than 1% of the administered dose. Therefore, neither TI-435 nor its metabolites appeared to exhibit potential for bioaccumulation.

Toxicologically significant compound(s): The major metabolites in both urine and feces were the parent compound (TI-435) and TZNG [N-(2-chlorothiazol-5-methyl)-N′-nitroguanidine], which resulted from N-demethylation of TI-435.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES—TECHNICAL GRADE CLOTHIANIDIN			
Oral in a 5% aqueous solution of arabic gum	Crl:CD.BR, rats (5/sex) 1758, 2283, 2965, 3850 or 5000 mg/kg bw	**LD_{50} males and females > 5000 mg/kg bw**	Clinical signs at all dose levels included ataxia, tremor, palpebral closure and hunched posture (1 hour–2 days).
Oral in a 5% aqueous solution of arabic gum	Crl:CD-1(ICR)BR, mice 304, 380, 475, 594 or 742 mg/kg bw	**LD_{50} males = 389 mg/kg bw** **LD_{50} females = 465 mg/kg bw**	4/10, 8/10, 8/10 and 10/10 animals died (2 hour–2 days) when dosed at 380, 475, 594, and 742 mg/kg bw TI-435. Clinical signs of reaction to treatment at all dose levels were decreased activity, palpebral closure, ataxia and tremor. Other clinical signs included tachypnoea, lethargy, prone posture, twitching, breathing changes, and hypothermia. **HIGH Toxicity**

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Dermal	Crl:CD.BR, rats 24 hour dose of 2000 mg/kg bw	**Dermal LD_{50} males and females** **> 2000 mg/kg bw**	**No mortalities occurred at the limit dose.** No clinical signs were observed. **LOW Toxicity**
Inhalation	Crl:CD.BR rats (5/sex) 4.5 hours by head only, at concentrations of 0 or 5.538 mg/L (gravimetrically determined)	**LC_{50} males and females** **> 5.538 mg/L**	Clinical signs included ataxia, semi-closed eyes, hunched body posture and lethargy were observed following exposure until day 3. No mortalities occurred. **LOW Toxicity**
Skin irritation	Crl:NZW/Kbl.BR New Zealand White rabbits given 0.5 g	No dermal irritation or signs of toxicity during the course of the observation period. Erythema and Edema scores were 0 for all animals, for all times tested. **Non-irritating to the skin**	
Eye irritation	6 male New Zealand White rabbits	Slight chemosis and ocular discharge were apparent in all rabbits shortly after instillation of the test article. All conjunctival irritation reactions resolved within 24 hours of treatment. **Slightly irritating to the eyes**	
Skin sensitization (Magnusson–Kligman Maximization)	Hartley guinea pigs tested at 20% and 10%		Negative
ACUTE STUDIES—FORMULATION [Poncho 600]			
Oral in demineralized water	Wistar rats (HsdCpb:WU) 200, 500 or 2000 mg/kg bw	**LD_{50} > 500 mg/kg bw and** **< 1000 mg/kg bw**	Decreased reactivity, spasmodic state, laboured breathing, closed eyelids and transient tremor. **WARNING—POISON**
Oral in demineralized water	Crl:WI(HAN)BR, rats (6/sex) 200, 500 or 2000 mg/kg bw	**LD_{50} males > 2000 mg/kg and LD_{50} females = 2000 mg/kg bw** **Supplemental** The formulation tested differed from the Poncho 600 formulation proposed for registration and as such the results of this study were not used for regulatory purposes.	
Dermal	Wistar rats (HsdCpb:WU) 5/sex 4000 mg/kg bw		All animals survived the observation period without treatment-related clinical signs.
Inhalation	Wistar rats (HsdCpb:WU) 5/sex 2.6 mg/L	**LC_{50} > 2.6 mg/L**	No mortalities occurred. Treatment-related bradypnea, laboured breathing, piloerection and ungroomed appearance occurred in all animals (0–3 days in males).
Skin irritation	Male Himalayan rabbits administered 0.5 mL		There were no clinical signs of toxicity. No cutaneous erythema or edema occurred in any animal, at any time point.

Appendix III Toxicology summary table

METABOLISM: RAT

Rate and extent of absorption and excretion: TI-435 was readily absorbed and excreted within 96 hours following a single 2.5 mg/kg bw or repeated oral dose of 25 mg/kg bw. At a dose of 250 mg/kg bw, absorption became biphasic and was saturated. Urinary excretion accounted for 89.2–94.6% of the administered radioactivity. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups (3.8–8.6%).

Distribution/target organ(s): There was a rapid absorption and distribution of administered radioactivity to all organs and tissues, followed by rapid excretion with reduction to background levels in most tissues and organs within 24 hours. Gender-related differences in plasma kinetics were indicated by the somewhat greater rate of absorption and elimination in females. Since tissue burdens were very low, neither TI-435 nor its metabolites appear to undergo significant sequestration.

Toxicologically significant compound(s): The metabolites identified (primarily oxidative demethylation products and cleavage products of the nitrogen-carbon bond between the nitroimino and thiazolyl moieties) were consistent with Phase I processes. Major metabolites found in composite urine samples were TZNG (7–12.5%), MNG (7.8–13.2%) and NTG (1.4–3.9% of administered).

METABOLISM: MICE

Rate and extent of absorption and excretion: TI-435 was readily absorbed and excreted within 168 hours following a single oral dose of 5 mg/kg bw. Urine was the major route of excretion of TI-435, accounting for 92.4–93.7% of the administered radioactivity. Feces accounted for 5.0–6.8% of the administered radioactivity. Within 24 hours, 89.0–91.7% of the administered radioactivity was excreted in the urine and 4.9–6.2% was excreted in the feces.
Distribution/target organ(s): Residual radioactivity in any given tissue at 168 hours post-dose was considerably less than 1% of the administered dose. Therefore, neither TI-435 nor its metabolites appeared to exhibit potential for bioaccumulation.
Toxicologically significant compound(s): The major metabolites in both urine and feces were the parent compound (TI-435) and TZNG [N-(2-chlorothiazol-5-methyl)-N'-nitroguanidine], which resulted from N-demethylation of TI-435.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES—TECHNICAL GRADE CLOTHIANIDIN			
Oral in a 5% aqueous solution of arabic gum	Crl:CD BR, rats (5/sex) 1758, 2283, 2965, 3850 or 5000 mg/kg bw	**LD$_{50}$ males and females > 5000 mg/kg bw**	Clinical signs at all dose levels included ataxia, tremor, palpebral closure and hunched posture (1 hour–2 days).
Oral in a 5% aqueous solution of arabic gum	Crl:CD-1(ICR)BR, mice 304, 380, 475, 594 or 742 mg/kg bw	**LD$_{50}$ males = 389 mg/kg bw** **LD$_{50}$ females = 465 mg/kg bw**	4/10, 8/10, 8/10 and 10/10 animals died (2 hour–2 days) when dosed at 380, 475, 594, and 742 mg/kg bw TI-435. Clinical signs of reaction to treatment at all dose levels were decreased activity, palpebral closure, ataxia and tremor. Other clinical signs included tachypnoea, lethargy, prone posture, twitching, breathing changes, and hypothermia. **HIGH Toxicity**

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Dermal	Crl:CD.BR, rats 24 hour dose of 2000 mg/kg bw	**Dermal LD_{50} males and females > 2000 mg/kg bw**	**No mortalities occurred at the limit dose.** No clinical signs were observed. **LOW Toxicity**
Inhalation	Crl:CD.BR rats (5/sex) 4.5 hours by head only, at concentrations of 0 or 5.538 mg/L (gravimetrically determined)	**LC_{50} males and females > 5.538 mg/L**	Clinical signs included ataxia, semi-closed eyes, hunched body posture and lethargy were observed following exposure until day 3. No mortalities occurred. **LOW Toxicity**
Skin irritation	Crl:NZW/Kbl.BR New Zealand White rabbits given 0.5 g	No dermal irritation or signs of toxicity during the course of the observation period. Erythema and Edema scores were 0 for all animals, for all times tested. **Non-irritating to the skin**	
Eye irritation	6 male New Zealand White rabbits	Slight chemosis and ocular discharge were apparent in all rabbits shortly after instillation of the test article. All conjunctival irritation reactions resolved within 24 hours of treatment. **Slightly irritating to the eyes**	
Skin sensitization (Magnusson–Kligman Maximization)	Hartley guinea pigs tested at 20% and 10%		Negative
ACUTE STUDIES—FORMULATION [Poncho 600]			
Oral in demineralized water	Wistar rats (HsdCpb:WU) 200, 500 or 2000 mg/kg bw	**$LD_{50} > 500$ mg/kg bw and < 1000 mg/kg bw**	Decreased reactivity, spasmodic state, laboured breathing, closed eyelids and transient tremor **WARNING—POISON**
Oral in demineralized water	Crl:WI(HAN)BR, rats (6/sex) 200, 500 or 2000 mg/kg bw	**LD_{50} males > 2000 mg/kg and LD_{50} females $= 2000$ mg/kg bw** **Supplemental** The formulation tested differed from the Poncho 600 formulation proposed for registration and as such the results of this study were not used for regulatory purposes.	
Dermal	Wistar rats (HsdCpb:WU) 5/sex 4000 mg/kg bw		All animals survived the observation period without treatment-related clinical signs.
Inhalation	Wistar rats (HsdCpb:WU) 5/sex 2.6 mg/L	**$LC_{50} > 2.6$ mg/L**	No mortalities occurred. Treatment-related bradypnea, laboured breathing, piloerection and ungroomed appearance occurred in all animals (0–3 days in males).
Skin irritation	Male Himalayan rabbits administered 0.5 mL		There were no clinical signs of toxicity. No cutaneous erythema or edema occurred in any animal, at any time point.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Eye irritation	Male Himalayan rabbits administered 0.1 mL		Scores for corneal opacity, iridial damage and conjunctival erythema and chemosis were 0 for all animals at all time points.
Skin sensitization (Buehler patch test)	Guinea pigs (Hsd Poc:DH), induction and challenge doses of 0.5 mL		Negative
ACUTE STUDIES—METABOLITES AND INTERMEDIATES			
Oral administered in aqueous carboxymethyl cellulose	HsdCpb:WU Wistar, rats (3/sex) given TI-435-CCMT-Adduct at 2000 mg/kg bw	Intermediate	$LD_{50} > 2000$ mg/kg bw
Oral in 2% aqueous Cremophor EL	HsdCpb:WU Wistar, rats (3/sex) were given TI-435-Hexahydropyrimidine (99.5%) at a dose of 2000 mg/kg bw	Intermediate	$LD_{50} > 2000$ mg/kg bw no clinical signs of toxicity
Oral in 5% aqueous arabic gum	Crl:CD.BR, rats (5/sex) were given methyl guanidine (MG) (98.8% a.i.) at doses of 260, 355, 435, 530 or 650 mg/kg bw	Metabolite	LD_{50} males = 550 mg/kg bw LD_{50} females = 446 mg/kg bw
Oral in polyethylene glycol 400	HsdCpb:WU Wistar, rats (3/sex) were given TI-435-Triazan (95.6%) at a dose of 2000 mg/kg bw	Intermediate	$LD_{50} > 2000$ mg/kg bw No clinical signs of toxicity were observed.
Oral in a 5% aqueous solution of arabic gum	Crl:CD.BR, rats (5/sex) were given TZMU (99.3% metabolite) at doses of 920, 1152, 1440, 1800 or 2250 mg/kg bw	Oral LD_{50} males = 1424 mg/kg bw Oral LD_{50} females = 1282 mg/kg bw Deaths occurred between 4.5 hours and 2 days following dosing. Clinical signs of toxicity included low gait, hunched posture, piloerection, ataxia, pronation, flaccidity, vasodilation and breathing irregularities.	
Oral in a 5% aqueous solution of arabic gum	Crl:CD.BR, rats (5/sex) were given TI-435 metabolite TZNG at doses of 1125, 1350 or 1450 mg/kg bw		Oral LD_{50} males > 1450 mg/kg bw Oral LD_{50} females = 1481 mg/kg bw Deaths occurred between 2 and 5 days following dosing, clinical signs of toxicity included lethargy, palpebral closure, arched gait, hunched posture and piloerection.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Skin irritation	Himalayan rabbits were dermally exposed to 0.5g of TI-435-CCMT-Adduct (99.4%)	Intermediate	No dermal irritation or signs of toxicity were observed.
Oral in a 5% aqueous solution of arabic gum	Crl:CD.BR, rats (5/group) were given a single oral gavage dose of TI-435 metabolite TMG (98.6% a.i.) at doses of 225, 550, 650, or 1100 mg/kg bw		Oral LD_{50} males < 550 mg/kg bw Oral LD_{50} females = 567 mg/kg bw Deaths occurred from 1 hour of dosing until day 2. Clinical signs included lethargy, palpebral closure, tremors, twitching, laboured or rapid breathing and prone body position.
Oral in corn oil	Crl:CD.BR, rats (5/sex) were given a single oral gavage dose of TI-435 metabolite BN0230M (94.4% a.i.) a dose of 2000 mg/kg bw		Oral LD_{50} males > 2000 mg/kg bw Oral LD_{50} females > 2000 mg/kg bw Males showed breathing changes, between ¼ and 1¾ hours of dosing.
Oral in a 5% aqueous solution of arabic gum	Crl:CD(SD)IGS.BR, rats (5/sex) were given a single oral dose of BN0335E2, a dose of 2000 mg/kg bw		Oral LD_{50} males > 2000 mg/kg bw Oral LD_{50} females > 2000 mg/kg bw No mortalities or clinical signs of toxicity were observed.
Skin irritation	Himalayan rabbits were dermally exposed to 0.5 g of TI-435-Triazan (95.6%)	Intermediate	No dermal irritation or signs of toxicity were observed. MAS = 0
Eye irritation	Himalayan rabbits eye instilled with 100 mg dose of TI-435-Triazan (95.6%)	Intermediate	TI-435-Triazan caused no ocular reactions at any time.
Eye irritation	Himalayan rabbits eye instilled with 100 mg dose of TI-435-CCMT-Adduct (99.4%)	Intermediate	TI-435-CCMT-Adduct caused no ocular reactions at any time.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Eye irritation	Male Himalayan rabbits administered 0.1 mL		Scores for corneal opacity, iridial damage and conjunctival erythema and chemosis were 0 for all animals at all time points.
Skin sensitization (Buehler patch test)	Guinea pigs (Hsd Poc:DH), induction and challenge doses of 0.5 mL		Negative
ACUTE STUDIES—METABOLITES AND INTERMEDIATES			
Oral administered in aqueous carboxymethyl cellulose	HsdCpb:WU Wistar, rats (3/sex) given TI-435-CCMT-Adduct at 2000 mg/kg bw	Intermediate	LD_{50} > 2000 mg/kg bw
Oral in 2% aqueous Cremophor EL	HsdCpb:WU Wistar, rats (3/sex) were given TI-435-Hexahydropyrimidine (99.5%) at a dose of 2000 mg/kg bw	Intermediate	LD_{50} > 2000 mg/kg bw no clinical signs of toxicity
Oral in 5% aqueous arabic gum	Crl:CD.BR, rats (5/sex) were given methyl guanidine (MG) (98.8% a.i.) at doses of 260, 355, 435, 530 or 650 mg/kg bw	Metabolite	LD_{50} males = 550 mg/kg bw LD_{50} females = 446 mg/kg bw
Oral in polyethylene glycol 400	HsdCpb:WU Wistar, rats (3/sex) were given TI-435-Triazan (95.6%) at a dose of 2000 mg/kg bw	Intermediate	LD_{50} > 2000 mg/kg bw No clinical signs of toxicity were observed.
Oral in a 5% aqueous solution of arabic gum	Crl:CD.BR, rats (5/sex) were given TZMU (99.3% metabolite) at doses of 920, 1152, 1440, 1800 or 2250 mg/kg bw	Oral LD_{50} males = 1424 mg/kg bw Oral LD_{50} females = 1282 mg/kg bw Deaths occurred between 4.5 hours and 2 days following dosing. Clinical signs of toxicity included low gait, hunched posture, piloerection, ataxia, pronation, flaccidity, vasodilation and breathing irregularities.	
Oral in a 5% aqueous solution of arabic gum	Crl:CD.BR, rats (5/sex) were given TI-435 metabolite TZNG at doses of 1125, 1350 or 1450 mg/kg bw		Oral LD_{50} males > 1450 mg/kg bw Oral LD_{50} females = 1481 mg/kg bw Deaths occurred between 2 and 5 days following dosing, clinical signs of toxicity included lethargy, palpebral closure, arched gait, hunched posture and piloerection.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Skin irritation	Himalayan rabbits were dermally exposed to 0.5g of TI-435-CCMT-Adduct (99.4%)	Intermediate	No dermal irritation or signs of toxicity were observed.
Oral in a 5% aqueous solution of arabic gum	Crl:CD.BR, rats (5/group) were given a single oral gavage dose of TI-435 metabolite TMG (98.6% a.i.) at doses of 225, 550, 650, or 1100 mg/kg bw		Oral LD$_{50}$ males < 550 mg/kg bw Oral LD$_{50}$ females = 567 mg/kg bw Deaths occurred from 1 hour of dosing until day 2. Clinical signs included lethargy, palpebral closure, tremors, twitching, laboured or rapid breathing and prone body position.
Oral in corn oil	Crl:CD.BR, rats (5/sex) were given a single oral gavage dose of TI-435 metabolite BN0230M (94.4% a.i.) a dose of 2000 mg/kg bw		Oral LD$_{50}$ males > 2000 mg/kg bw Oral LD$_{50}$ females > 2000 mg/kg bw Males showed breathing changes, between ¼ and 1¾ hours of dosing.
Oral in a 5% aqueous solution of arabic gum	Crl:CD(SD)IGS.BR, rats (5/sex) were given a single oral dose of BN0335E2, a dose of 2000 mg/kg bw		Oral LD$_{50}$ males > 2000 mg/kg bw Oral LD$_{50}$ females > 2000 mg/kg bw No mortalities or clinical signs of toxicity were observed.
Skin irritation	Himalayan rabbits were dermally exposed to 0.5 g of TI-435-Triazan (95.6%)	Intermediate	No dermal irritation or signs of toxicity were observed. MAS = 0
Eye irritation	Himalayan rabbits eye instilled with 100 mg dose of TI-435-Triazan (95.6%)	Intermediate	TI-435-Triazan caused no ocular reactions at any time.
Eye irritation	Himalayan rabbits eye instilled with 100 mg dose of TI-435-CCMT-Adduct (99.4%)	Intermediate	TI-435-CCMT-Adduct caused no ocular reactions at any time.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
SHORT-TERM AND CHRONIC TOXICITY (RAT)			
28-day dermal	Crl:CD(SD)IGS BR rats (10/sex/group) at 0, 100, 300, or 1000 mg/kg bw/d	**LOAEL males = 1000 mg/kg bw/d** **NOAEL males = 300 mg/kg bw/d** **NOAEL females = 1000 mg/kg bw/d**	1000 mg/kg bw: ↓ bwg (males)
28-day dietary	Crl:CD BR rats (5/sex/group) 0, 1250, 2500, 5000, or 7500 ppm (equivalent to 120/137, 249/228, 475/454, 602/689 mg/kg bw/d [males/females])	**LOAEL = 2500 ppm (249/228 mg/kg bw/d)** **NOAEL = 1250 ppm (120/137 mg/kg bw/d)**	≥ 2500 ppm: ↓ FC, FE and bwg (males/females) ≥ 5000 ppm: half-closed eyes, small thymus, ↓ medullary cellularity (thymus) (males/females); ↓ reticulocytes, neutrophils and WBC, ↑ blood urea nitrogen, testicular atrophy, ↓ cellularity of white pulp (spleen) (males); ↓ thymus wt, ↑ ALT (females) 7500 ppm: brown nasal staining, involution (thymus), ↑ AST (males/females); ↓ lymphocytes, basophils, monocytes, eosinophils and leucocytes, ↓ thymus wt (males); ↓ reticulocytes, ↑ blood urea nitrogen (females)
90-day dietary	Sprague-Dawley rats (10/sex/group) 0, 100, 250, 1250 or 2500 ppm (equivalent to 7.7/9.4, 19.7/24.0, 96/119 and 189/232 mg/kg bw/d [males/females])	**NOAEL/LOAEL not established**	≥ 250ppm: ↓ WBC (males) ≥ 1250 ppm: ↓ bwg, ↑ spleen, lung, uterus and ovary wt (females) ≥ 2500 ppm: ↓ FC, and bwg, ↑ liver wt (males/females); ↑ adrenal and heart wt (females) **Supplemental**
90-day dietary	Sprague-Dawley rats (15/sex/group) 0, 150, 500, or 3000 ppm (equivalent to 9.0/10.9, 27.9/34.0, and 202.0/254.2 mg/kg bw/d [males/females])	**LOAEL = 3000 ppm (202/254.2 mg/kg bw/d)** **NOAEL = 500 ppm (27.9/34.0 mg/kg bw/d)**	3000 ppm: ↓ bw and bwg (males/females); N-demethylase (↑ 32%), o-demethylase ↑ 50%, ethoxyresorufin o-deethylase ↑ 127%, and pentoxyresorufin o-dealkylase ↑ 211% (males)

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
2-year dietary	Crl:CD®(SD)BR VAF/Plus® rats (80/sex/group) 0, 150, 500, 1500 or 3000 ppm (equivalent to 0/0, 8.1/9.7, 27.4/32.5, 82.0/97.8, and 156.5/193.4 mg/kg bw/d [males/females])	**LOAEL = 1500 ppm (82.0/97.8 mg/kg bw/d)** **NOAEL = 500 ppm (27.4/32.5 mg/kg bw/d)**	≥ 1500 ppm: ↑ liver congestion and granular material in kidneys (males); ↓ bw and FC, ↑ altered hepatocellular eosinophilic focus, ↑ liver lymphohistiocytic infiltrate, ↑ ovary interstitial gland hyperplasia (females) 3000 ppm: ↓ bw and FC, ↑ pelvic mineralization, calculus and transitional cell hyperplasia, mottled livers, ↑ altered hepatocellular eosinophilic focus, ↑ stomach edema and hemorrhage (males); ↑ pelvic angiectasis and tubular ectasia, ↑ kidney wt, liver wt and lung wt, ↑ lung inflammation, ↑ thymus epithelial hyperplasia, ↑ stomach glandular erosion and edema, ↑ uterus congestion, ↑ pituitary angiectasis, ↑ thyroid follicular cysts (females) ≥ 1500 ppm: ↑ thyroid C-cell adenoma (females) 7(9%), 13(16%), 9(11%), 17**(21%), 16**(20%) (h.c. 8.8–12%) ** = $p < 0.05$
SHORT-TERM AND CHRONIC TOXICITY (DOG)			
28-day dietary	Beagle dogs (3/sex/group) 0, 1250, 2500, or 5000 ppm (equivalent to 0/0, 34.3/35.8, 36.9/53.5, 62.4/57.4 mg/kg bw/d [males/females])	**LOAEL = 2500 ppm (36.9/53.5 mg/kg bw/d)** **NOAEL = 1250 ppm (34.3/35.8 mg/kg bw/d)**	≥ 2500 ppm: mortalities (2/6), ↓ FC and bwg ↓ leucocytes, ↓ lymphocytes, ↓ neutrophils, ↓ platelet, ↓ ALT, ↓ protein, ↓ albumin, anemia, BM congestion, hypocellularity and hemorrhage, depleted lymph of the spleen, ileum, thymus and mesenteric lymph nodes (males/females) 5000 ppm: total mortality
90-day dietary	Beagle dogs (4/sex/group) 0, 325, 650, 1500 or 2250 ppm (0/0, 9.2/9.6, 19.3/21.2, 40.9/42.1 and 58.2/61.8 mg/kg bw/d [males/females])	**LOAEL = 1500 ppm (40.9 mg/kg bw/d) (males)** **LOAEL = 2250 ppm (61.8 mg/kg bw/d) (females)** **NOAEL = 650 ppm (19.3 mg/kg bw/d) (males)** **NOAEL = 1500 ppm (42.1 mg/kg bw/d) (females)**	≥ 1500 ppm: thinness, ↓ bw and bwg, anemia (males) 2250 ppm: ↓ lymphocytes, ↓ WBC, ↓ ALT, ↓ neutrophils, ↓ total protein and ↓ albumin (females)

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
SHORT-TERM AND CHRONIC TOXICITY (RAT)			
28-day dermal	Crl:CD(SD)IGS BR rats (10/sex/group) at 0, 100, 300, or 1000 mg/kg bw/d	**LOAEL males = 1000 mg/kg bw/d** **NOAEL males = 300 mg/kg bw/d** **NOAEL females = 1000 mg/kg bw/d**	1000 mg/kg bw: ↓ bwg (males)
28-day dietary	Crl:CD BR rats (5/sex/group) 0, 1250, 2500, 5000, or 7500 ppm (equivalent to 120/137, 249/228, 475/454, 602/689 mg/kg bw/d [males/females])	**LOAEL = 2500 ppm (249/228 mg/kg bw/d)** **NOAEL = 1250 ppm (120/137 mg/kg bw/d)**	≥ 2500 ppm: ↓ FC, FE and bwg (males/females) ≥ 5000 ppm: half-closed eyes, small thymus, ↓ medullary cellularity (thymus) (males/females); ↓ reticulocytes, neutrophils and WBC, ↑ blood urea nitrogen, testicular atrophy, ↓ cellularity of white pulp (spleen) (males); ↓ thymus wt, ↑ ALT (females) 7500 ppm: brown nasal staining, involution (thymus), ↑ AST (males/females); ↓ lymphocytes, basophils, monocytes, eosinophils and leucocytes, ↓ thymus wt (males); ↓ reticulocytes, ↑ blood urea nitrogen (females)
90-day dietary	Sprague-Dawley rats (10/sex/group) 0, 100, 250, 1250 or 2500 ppm (equivalent to 7.7/9.4, 19.7/24.0, 96/119 and 189/232 mg/kg bw/d [males/females])	**NOAEL/LOAEL not established**	≥ 250ppm: ↓ WBC (males) ≥ 1250 ppm: ↓ bwg, ↑ spleen, lung, uterus and ovary wt (females) ≥ 2500 ppm: ↓ FC, and bwg, ↑ liver wt (males/females); ↑ adrenal and heart wt (females) **Supplemental**
90-day dietary	Sprague-Dawley rats (15/sex/group) 0, 150, 500, or 3000 ppm (equivalent to 9.0/10.9, 27.9/34.0, and 202.0/254.2 mg/kg bw/d [males/females])	**LOAEL = 3000 ppm (202/254.2 mg/kg bw/d)** **NOAEL = 500 ppm (27.9/34.0 mg/kg bw/d)**	3000 ppm: ↓ bw and bwg (males/females); N-demethylase (↑ 32%), o-demethylase ↑ 50%, ethoxyresorufin o-deethylase ↑ 127%, and pentoxyresorufin o-dealkylase ↑ 211% (males)

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
2-year dietary	Crl:CD®(SD)BR VAF/Plus® rats (80/sex/group) 0, 150, 500, 1500 or 3000 ppm (equivalent to 0/0, 8.1/9.7, 27.4/32.5, 82.0/97.8, and 156.5/193.4 mg/kg bw/d [males/females])	**LOAEL = 1500 ppm (82.0/97.8 mg/kg bw/d)** **NOAEL = 500 ppm (27.4/32.5 mg/kg bw/d)**	≥ 1500 ppm: ↑ liver congestion and granular material in kidneys (males); ↓ bw and FC, ↑ altered hepatocellular eosinophilic focus, ↑ liver lymphohistiocytic infiltrate, ↑ ovary interstitial gland hyperplasia (females) 3000 ppm: ↓ bw and FC, ↑ pelvic mineralization, calculus and transitional cell hyperplasia, mottled livers, ↑ altered hepatocellular eosinophilic focus, ↑ stomach edema and hemorrhage (males); ↑ pelvic angiectasis and tubular ectasia, ↑ kidney wt, liver wt and lung wt, ↑ lung inflammation, ↑ thymus epithelial hyperplasia, ↑ stomach glandular erosion and edema, ↑ uterus congestion, ↑ pituitary angiectasis, ↑ thyroid follicular cysts (females) ≥ 1500 ppm: ↑ thyroid C-cell adenoma (females) 7(9%), 13(16%), 9(11%), 17**(21%), 16**(20%) (h.c. 8.8–12%) ** = $p < 0.05$
SHORT-TERM AND CHRONIC TOXICITY (DOG)			
28-day dietary	Beagle dogs (3/sex/group) 0, 1250, 2500, or 5000 ppm (equivalent to 0/0, 34.3/35.8, 36.9/53.5, 62.4/57.4 mg/kg bw/d [males/females])	**LOAEL = 2500 ppm (36.9/53.5 mg/kg bw/d)** **NOAEL = 1250 ppm (34.3/35.8 mg/kg bw/d)**	≥ 2500 ppm: mortalities (2/6), ↓ FC and bwg ↓ leucocytes, ↓ lymphocytes, ↓ neutrophils, ↓ platelet, ↓ ALT, ↓ protein, ↓ albumin, anemia, BM congestion, hypocellularity and hemorrhage, depleted lymph of the spleen, ileum, thymus and mesenteric lymph nodes (males/females) 5000 ppm: total mortality
90-day dietary	Beagle dogs (4/sex/group) 0, 325, 650, 1500 or 2250 ppm (0/0, 9.2/9.6, 19.3/21.2, 40.9/42.1 and 58.2/61.8 mg/kg bw/d [males/females])	**LOAEL = 1500 ppm (40.9 mg/kg bw/d) (males)** **LOAEL = 2250 ppm (61.8 mg/kg bw/d) (females)** **NOAEL = 650 ppm (19.3 mg/kg bw/d) (males)** **NOAEL = 1500 ppm (42.1 mg/kg bw/d) (females)**	≥ 1500 ppm: thinness, ↓ bw and bwg, anemia (males) 2250 ppm: ↓ lymphocytes, ↓ WBC, ↓ ALT, ↓ neutrophils, ↓ total protein and ↓ albumin (females)

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
12-month dietary	Beagle dogs (4/sex/group) 0, 325, 650, 1500, or 2000 ppm (0/0, 4.8/8.5, 16.6/15.0, 36.3/40.1 and 46.4/52.9 mg/kg bw/d [males/females])	**LOAEL = 2000 ppm (52.9 mg/kg bw/d) (females)** **NOAEL = 2000 ppm (46.4 mg/kg bw day) (males)** **NOAEL = 1500 ppm 40.1 mg/kg bw/d) (females)**	≥ 1500 ppm: ↓ (ALT) (males/females) (non-adverse) 2000 ppm: anemia (females)
SHORT-TERM AND CHRONIC TOXICITY (MICE)			
28-day dietary	Crl:CD-1(ICR)BR mice(6/sex/group) 0, 500, 1000, 2000, or 4000 ppm (0, 90/122, 190/248, 383/491 and 683/619 mg/kg bw/d [males/females])	**LOAEL = 500 ppm (90/122 mg/kg bw/d)** **NOAEL not established**	≥ 500 ppm: ↓ FC (males/females), ↑ lung wt (females) ≥ 1000 ppm: ↓ FE (males/females), ↑ neutrophils (males) ≥ 2000 ppm: thin appearance and hunched posture, ↓ bw and ↓ bwg, ↓ liver, spleen and kidney wts., ↑ adrenal wt (males/females); mortality, ↑ AST, ↓ lung and heart wts., ↑ pituitary wt (males); ↑ eosinophils, ↑ ALT, ↓ glucose, ↓ triglycerides, atrophy of the red pulp of the spleen, ↓ pituitary, thymus and ovary wts., ↑ thyroid and lung wts. (females) 4000 ppm: mortality, lethargy, body tremors, unsteady gait, spleen abnormalities (males/females); ↓ PCV, Hgb, RBC, MCV and lymphocytes, ↓ colloid in prostate and seminal vesicles, testicular atrophy (males); congested lungs and adrenals, distended gall bladders, small, pale spleens (females)
90-day dietary	Crl:CD-1(ICR)BR mice (10/sex/group) 0, 100, 500, 1000 or 1500 ppm (0, 16/22, 82/107, 160/207 and 263/329 mg/kg bw/d [males/females])	**NOAEL/LOAEL not established**	≥ 500 ppm: ↓ kidney wt (males); adverse biochemistry (females) ≥ 1000 ppm: ↓ bwg (males/females); ↓ kidney wt (females) ≥ 1500 ppm: ↑ vocalizations (males/females); ↓ lung wt, ↑ incidence of sparse corpora lutea and follicles, ↑ prominence of endometrial glands (females); ↑ epididymal wt (males) **Supplemental**

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
78-week dietary	Crl:CD-1®(ICR)BR VAF/Plus® mice (50/sex/group) 0, 100, 350, 1250 or 2000/1800 ppm (equivalent to 0, 13.5/17.0, 47.2/65.1, 171.4/215.9 or 254.1/322.3 mg/kg bw/d [males/females])	**LOAEL = 1250 ppm (171.4/215.9 mg/kg bw/d)** **NOAEL = 350 ppm (47.2/65.1 mg/kg bw/d)**	≥ 1250 ppm: ↑ vocalizations (males/females); ↓ bw and bwg (females) 2000/1800 ppm: ↓ FC and FE (males/females); ↓ bw, bwg (males); ↓ survival, ↑ pulmonary and adrenal cortex congestion, ↑ cervix fibromuscular hyperplasia (females)
REPRODUCTION AND DEVELOPMENTAL TOXICITY			
1-generation reproduction	Sprague-Dawley rats (20/sex/group) 0, 50, 100, 500 and 1000 ppm (0, 2.9/3.4, 5.8/6.6, 29.1/34.2 and 58.9/68.6 mg/kg bw/d [males/females])	**No LOAEL established** **NOAEL = 1000 ppm (58.9/68.6 mg/kg bw/d) (males/females)**	No treatment-related effects were observed.
Multigeneration	Sprague-Dawley rats (30/sex/group) 0, 150, 500 or 2500 ppm	**Parental LOAEL = 2500 ppm (163.4/188.8 mg/kg bw/d) (males/females)** **Offspring toxicity LOAEL = 500 ppm (31.2/36.8 mg/kg bw/d) (males/females)** **NOAEL = 150 ppm (9.8/11.5 mg/kg bw/d) (males/females)**	**Parental toxicity** 2500 ppm: (males/females, F_0 and F_1) ↓ bwg, bw, FE, ↓ thymus weight **Offspring toxicity** ≥ 500 ppm: ↓ thymus and ↓ bwg (F_1), delayed sexual maturation (males); ↑ stillbirths 2500 ppm: ↓ bwg, ↓ thymus (males/females, F_1 and F_2); ↓ spleen weight (females, F_1; males/females, F_2); delayed sexual maturation (males/females) **Reproductive** 2500 ppm: ↓ % progressively motile sperm in high-dose (F_0/F_1); ↓ % motile sperm (F_1), ↑ % sperm with detached heads (F_0/F_1)

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
12-month dietary	Beagle dogs (4/sex/group) 0, 325, 650, 1500, or 2000 ppm (0/0, 4.8/8.5, 16.6/15.0, 36.3/40.1 and 46.4/52.9 mg/kg bw/d [males/females])	**LOAEL = 2000 ppm (52.9 mg/kg bw/d) (females)** **NOAEL = 2000 ppm (46.4 mg/kg bw day) (males)** **NOAEL = 1500 ppm 40.1 mg/kg bw/d) (females)**	≥ 1500 ppm: ↓ (ALT) (males/females) (non-adverse) 2000 ppm: anemia (females)
SHORT-TERM AND CHRONIC TOXICITY (MICE)			
28-day dietary	Crl:CD-1(ICR)BR mice(6/sex/group) 0, 500, 1000, 2000, or 4000 ppm (0, 90/122, 190/248, 383/491 and 683/619 mg/kg bw/d [males/females])	**LOAEL = 500 ppm (90/122 mg/kg bw/d)** **NOAEL not established**	≥ 500 ppm: ↓ FC (males/females), ↑ lung wt (females) ≥ 1000 ppm: ↓ FE (males/females), ↑ neutrophils (males) ≥ 2000 ppm: thin appearance and hunched posture, ↓ bw and ↓ bwg, ↓ liver, spleen and kidney wts., ↑ adrenal wt (males/females); mortality, ↑ AST, ↓ lung and heart wts., ↑ pituitary wt (males); ↑ eosinophils, ↑ ALT, ↓ glucose, ↓ triglycerides, atrophy of the red pulp of the spleen, ↓ pituitary, thymus and ovary wts., ↑ thyroid and lung wts. (females) 4000 ppm: mortality, lethargy, body tremors, unsteady gait, spleen abnormalities (males/females); ↓ PCV, Hgb, RBC, MCV and lymphocytes, ↓ colloid in prostate and seminal vesicles, testicular atrophy (males); congested lungs and adrenals, distended gall bladders, small, pale spleens (females)
90-day dietary	Crl:CD-1(ICR)BR mice (10/sex/group) 0, 100, 500, 1000 or 1500 ppm (0, 16/22, 82/107, 160/207 and 263/329 mg/kg bw/d [males/females])	**NOAEL/LOAEL not established**	≥ 500 ppm: ↓ kidney wt (males); adverse biochemistry (females) ≥ 1000 ppm: ↓ bwg (males/females); ↓ kidney wt (females) ≥ 1500 ppm: ↑ vocalizations (males/females); ↓ lung wt, ↑ incidence of sparse corpora lutea and follicles, ↑ prominence of endometrial glands (females); ↑ epididymal wt (males) **Supplemental**

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
78-week dietary	Crl:CD-1®(ICR)BR VAF/Plus® mice (50/sex/group) 0, 100, 350, 1250 or 2000/1800 ppm (equivalent to 0, 13.5/17.0, 47.2/65.1, 171.4/215.9 or 254.1/322.3 mg/kg bw/d [males/females])	**LOAEL = 1250 ppm (171.4/215.9 mg/kg bw/d)** **NOAEL = 350 ppm (47.2/65.1 mg/kg bw/d)**	≥ 1250 ppm: ↑ vocalizations (males/females); ↓ bw and bwg (females) 2000/1800 ppm: ↓ FC and FE (males/females); ↓ bw, bwg (males); ↓ survival, ↑ pulmonary and adrenal cortex congestion, ↑ cervix fibromuscular hyperplasia (females)
REPRODUCTION AND DEVELOPMENTAL TOXICITY			
1-generation reproduction	Sprague-Dawley rats (20/sex/group) 0, 50, 100, 500 and 1000 ppm (0, 2.9/3.4, 5.8/6.6, 29.1/34.2 and 58.9/68.6 mg/kg bw/d [males/females])	**No LOAEL established** **NOAEL = 1000 ppm (58.9/68.6 mg/kg bw/d) (males/females)**	No treatment-related effects were observed.
Multigeneration	Sprague-Dawley rats (30/sex/group) 0, 150, 500 or 2500 ppm	**Parental LOAEL = 2500 ppm (163.4/188.8 mg/kg bw/d) (males/females)** **Offspring toxicity LOAEL = 500 ppm (31.2/36.8 mg/kg bw/d) (males/females)** **NOAEL = 150 ppm (9.8/11.5 mg/kg bw/d) (males/females)**	**Parental toxicity** 2500 ppm: (males/females, F_0 and F_1) ↓ bwg, bw, FE, ↓ thymus weight **Offspring toxicity** ≥ 500 ppm: ↓ thymus and ↓ bwg (F_1), delayed sexual maturation (males); ↑ stillbirths 2500 ppm: ↓ bwg, ↓ thymus (males/females, F_1 and F_2); ↓ spleen weight (females, F_1; males/females, F_2); delayed sexual maturation (males/females) **Reproductive** 2500 ppm: ↓ % progressively motile sperm in high-dose (F_0/F_1); ↓ % motile sperm (F_1), ↑ % sperm with detached heads (F_0/F_1)

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Dosage-range developmental toxicity by gavage (0.5% methyl cellulose)	Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats (8/group) 0, 125, 250, 500 or 1000 mg/kg bw/d from days 6 through 19 of gestation.	**Maternal toxicity LOAEL ≤ 125 mg/kg bw/d**	**Parental** ≥ 125 mg/kg bw/d: ↓ bw, FC, gravid uterine weights ≥ 250 mg/kg bw/d: ↑ early resorptions, ↓ litter size, ↑ % resorbed conceptuses, red perivaginal substance, scant feces ≥ 500mg/kg bw/d: total resorptions, ↓ motor activity, tremors, chromorhinorrhea, ptosis, cold to the touch, emaciation, dehydration, piloerection, staining, small spleens 1000 mg/kg bw/d: 8/8 mortality, dark urine **Developmental** ≥ 250 mg/kg bw/d: ↓ bw
Developmental toxicity by gavage (0.5% methyl cellulose)	Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats (25/group) 0, 10, 40 or 125 mg/kg bw/d from days 6 through 19 of gestation	**Maternal LOAEL = 40 mg/kg bw/d** **NOAEL = 10 mg/kg bw/d** **Developmental NOAEL ≥ 125 mg/kg bw/d**	**Parental** ≥ 40 mg/kg bw/d: ↓ bwg, FC 125 mg/kg bw/d: ↓ bw No developmental effects were observed. **Not teratogenic**
Dosage-range developmental toxicity (0.5% methyl cellulose)	New Zealand White [Hra:(NZW)SPF] rabbits (5/dose) 0, 62.5, 125, 250, or 500 mg/kg bw/d from days 6 through 28 of gestation	**Maternal toxicity LOAEL = 125 mg/kg bw/d** **NOAEL = 62.5 mg/kg bw/d**	**Parental** ≥ 125mg/kg bw/d: dark urine, complete mortalities, scant, absent or mucoid feces and/or red substance in the cage pan, ↓ bw, FC ≥ 250mg/kg bw/d: emaciation, laboured breathing, perinasal substance, and/or decreased motor activity 500mg/kg bw/d: lacrimation, impaired or lost righting reflex and/or head tilt only low-dose fetuses were available for examination due to losses at higher doses. No adverse effects noted.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Developmental toxicity 0.5% methyl cellulose by gavage	New Zealand White [Hra:(NZW)SPF] rabbits (23 females/dose) 0, 10, 25, 75, or 100 mg/kg bw/d from days 6 through 28 of gestation	**Maternal LOAEL = 75 mg/kg bw/d** **Maternal NOAEL = 25 mg/kg bw/d** **Developmental LOAEL = 75 mg/kg bw/d** **Developmental NOAEL = 25 mg/kg bw/d**	**Maternal** ≥ 75 mg/kg bw/d: scant feces, orange urine, mortality (2/23), ↓ bwg, FC 100 mg/kg bw/d: ↓ bw, mortality, early deliveries, red substance in cage pan **Developmental** ≥ 75 mg/kg bw/d: early delivery (2/23), ↓ gravid uterine weight, abortion, 100 mg/kg bw/d: abortions (6/23**), ↑ early and total resorptions, post implantation loss, (½3) complete resorptions **Fetal** ≥ 75 mg/kg bw/d: absent lung lobe, ↓ ossified sternal centre 100 mg/kg bw/d: ↓ bw, ↑ incomplete ossification of the sternal centra and absent hindpaw phalanges **Not teratogenic**

GENOTOXICITY

STUDY	SPECIES and STRAIN or CELL TYPE AND CONCENTRATIONS or DOSES	RESULTS
Gene mutations in bacteria	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; *E. Coli* WP2uvrA 100–5000 µg/plate with and without activation	Positive for TA1535 only with S9 activation
Salmonella/ microsome test	*Salmonella typhimurium* strains TA1535, TA100, TA1537, TA98 and TA102 from 16 to 5000 µg/plate with and without activation	Negative
Gene mutations in bacteria	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; *E. Coli* WP2uvrA 100–5000 µg/plate; with and without activation	Negative
In vitro mutagenicity DNA-repair	*Bacillus subtillis* from 375 to 6000 µg/disc with and without activation	Negative Supplemental: only 1 plate used for test, positive and negative controls.
In vivomammalian cytogenetics—micronucleus assay	CD-1 mice dosed with 25, 50 or 100 mg/kg active in peanut oil	Negative
Gene mutations in bacteria	*Salmonella typhimurium* strain TA1535 from 1000 to 8000 µg/plate with and without activation	Negative

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Dosage-range developmental toxicity by gavage (0.5% methyl cellulose)	Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats (8/group) 0, 125, 250, 500 or 1000 mg/kg bw/d from days 6 through 19 of gestation.	**Maternal toxicity LOAEL ≤ 125 mg/kg bw/d**	**Parental** ≥ 125 mg/kg bw/d: ↓ bw, FC, gravid uterine weights ≥ 250 mg/kg bw/d: ↑ early resorptions, ↓ litter size, ↑ % resorbed conceptuses, red perivaginal substance, scant feces ≥ 500mg/kg bw/d: total resorptions, ↓ motor activity, tremors, chromorhinorrhea, ptosis, cold to the touch, emaciation, dehydration, piloerection, staining, small spleens 1000 mg/kg bw/d: 8/8 mortality, dark urine **Developmental** ≥ 250 mg/kg bw/d: ↓ bw
Developmental toxicity by gavage (0.5% methyl cellulose)	Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats (25/group) 0, 10, 40 or 125 mg/kg bw/d from days 6 through 19 of gestation	**Maternal LOAEL = 40 mg/kg bw/d** **NOAEL = 10 mg/kg bw/d** **Developmental NOAEL ≥ 125 mg/kg bw/d**	**Parental** ≥ 40 mg/kg bw/d: ↓ bwg, FC 125 mg/kg bw/d: ↓ bw No developmental effects were observed. **Not teratogenic**
Dosage-range developmental toxicity (0.5% methyl cellulose)	New Zealand White [Hra:(NZW)SPF] rabbits (5/dose) 0, 62.5, 125, 250, or 500 mg/kg bw/d from days 6 through 28 of gestation	**Maternal toxicity LOAEL = 125 mg/kg bw/d** **NOAEL = 62.5 mg/kg bw/d**	**Parental** ≥ 125mg/kg bw/d: dark urine, complete mortalities, scant, absent or mucoid feces and/or red substance in the cage pan, ↓ bw, FC ≥ 250mg/kg bw/d: emaciation, laboured breathing, perinasal substance, and/or decreased motor activity 500mg/kg bw/d: lacrimation, impaired or lost righting reflex and/or head tilt only low-dose fetuses were available for examination due to losses at higher doses. No adverse effects noted.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Developmental toxicity 0.5% methyl cellulose by gavage	New Zealand White [Hra:(NZW)SPF] rabbits (23 females/dose) 0, 10, 25, 75, or 100 mg/kg bw/d from days 6 through 28 of gestation	**Maternal LOAEL = 75 mg/kg bw/d** **Maternal NOAEL = 25 mg/kg bw/d** **Developmental LOAEL = 75 mg/kg bw/d** **Developmental NOAEL = 25 mg/kg bw/d**	**Maternal** ≥ 75 mg/kg bw/d: scant feces, orange urine, mortality (2/23), ↓ bwg, FC 100 mg/kg bw/d: ↓ bw, mortality, early deliveries, red substance in cage pan **Developmental** ≥ 75 mg/kg bw/d: early delivery (2/23), ↓ gravid uterine weight, abortion, 100 mg/kg bw/d: abortions (6/23**), ↑ early and total resorptions, post implantation loss, (½3) complete resorptions **Fetal** ≥ 75 mg/kg bw/d: absent lung lobe, ↓ ossified sternal centre 100 mg/kg bw/d: ↓ bw, ↑ incomplete ossification of the sternal centra and absent hindpaw phalanges **Not teratogenic**

GENOTOXICITY

STUDY	SPECIES and STRAIN or CELL TYPE AND CONCENTRATIONS or DOSES	RESULTS
Gene mutations in bacteria	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; *E. Coli* WP2uvrA 100–5000 µg/plate with and without activation	Positive for TA1535 only with S9 activation
Salmonella/ microsome test	*Salmonella typhimurium* strains TA1535, TA100, TA1537, TA98 and TA102 from 16 to 5000 µg/plate with and without activation	Negative
Gene mutations in bacteria	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; *E. Coli* WP2uvrA 100–5000 µg/plate; with and without activation	Negative
In vitro mutagenicity DNA-repair	*Bacillus subtillis* from 375 to 6000 µg/disc with and without activation	Negative Supplemental: only 1 plate used for test, positive and negative controls.
In vivomammalian cytogenetics—micronucleus assay	CD-1 mice dosed with 25, 50 or 100 mg/kg active in peanut oil	Negative
Gene mutations in bacteria	*Salmonella typhimurium* strain TA1535 from 1000 to 8000 µg/plate with and without activation	Negative

STUDY	SPECIES and STRAIN or CELL TYPE AND CONCENTRATIONS or DOSES	RESULTS
Gene mutations in mammalian cells in vitro	Mouse L5178Y cells 0, 312.5, 625, 1250, 1667 and 2500 µg/mL (except 1, -S9) 0, 300, 600, 1200, 1600 and 2000 µg/mL (except 2, -S9) 0, 600, 1200, 1600, 2000 and 2400 µg/mL(+S9)	Positive
Unscheduled DNA synthesis (in vivo/in vitro)	Primary rat hepatocytes (male SD rats) 0, 2500, 5000 mg/kg (single oral dose; primary cultures scored for unscheduled DNA synthesis 2–4 and 12–16 hours after dose administration)	Negative
Chromosome aberrations in vitro	Chinese hamster lung cells 0, 39, 78.13, 156.25, 312.5,625, 937.5 and 1250 µg/mL without activation 0, 312.5, 625, 937.5, 1250, 1562.5 and 1875 µg/mL with activation	Positive with and without activation
Gene mutation in mammalian cells	Chinese hamster lung V79 cells 0, 156, 313, 625, 1250, 2500 and 5000 µg/mL	Negative
GENOTOXICITY METABOLITES IMPURITIES AND INTERMEDIATES		
Gene mutation bacterial reverse mutation assay BN0335E2 metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 1.6 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TZMU metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay methyl guanidine metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TZNG metabolite	*Salmonella typhimurium* strains(A98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TMG metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 µg/plate (in DMF) in the presence and absence of a metabolic activation	Negative

STUDY	SPECIES and STRAIN or CELL TYPE AND CONCENTRATIONS or DOSES	RESULTS
Gene mutation bacterial reverse mutation assay BN0230M metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 1.6 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay MAI metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay N-methylnitroguanidin metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 50 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TI 435-Triazan intermediate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 50 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TI 435-CCMT-Adduct intermediate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 16 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative

SPECIAL STUDIES

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
13-week subchronic neurotoxicity dietary	Fischer 344 rats (12/sex/group) 0, 150, 1000 or 3000 ppm (equivalent to 0/0, 9.2/10.6, 60.0/71.0 or 177.0/200.1 mg/kg bw/d	**LOAEL = 3000 ppm (177.0/200.1 mg/kg bw/d)** **NOAEL = 1000 ppm (60.0/71.0 mg/kg bw/d)**	3000 ppm: ↓ bw, FC and bwg (males/females) No evidence of neurotoxicity

STUDY	SPECIES and STRAIN or CELL TYPE AND CONCENTRATIONS or DOSES	RESULTS
Gene mutations in mammalian cells in vitro	Mouse L5178Y cells 0, 312.5, 625, 1250, 1667 and 2500 μg/mL (except 1, -S9) 0, 300, 600, 1200, 1600 and 2000 μg/mL (except 2, -S9) 0, 600, 1200, 1600, 2000 and 2400 μg/mL(+S9)	Positive
Unscheduled DNA synthesis (in vivo/in vitro)	Primary rat hepatocytes (male SD rats) 0, 2500, 5000 mg/kg (single oral dose; primary cultures scored for unscheduled DNA synthesis, 2–4 and 12–16 hours after dose administration)	Negative
Chromosome aberrations in vitro	Chinese hamster lung cells 0, 39, 78.13, 156.25, 312.5,625, 937.5 and 1250 μg/mL without activation 0, 312.5, 625, 937.5, 1250, 1562.5 and 1875 μg/mL with activation	Positive with and without activation
Gene mutation in mammalian cells	Chinese hamster lung V79 cells 0, 156, 313, 625, 1250, 2500 and 5000 μg/mL	Negative
GENOTOXICITY METABOLITES IMPURITIES AND INTERMEDIATES		
Gene mutation bacterial reverse mutation assay BN0335E2 metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 1.6 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TZMU metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay methyl guanidine metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TZNG metabolite	*Salmonella typhimurium* strains(A98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 μg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TMG metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 μg/plate (in DMF) in the presence and absence of a metabolic activation	Negative

STUDY	SPECIES and STRAIN or CELL TYPE AND CONCENTRATIONS or DOSES	RESULTS
Gene mutation bacterial reverse mutation assay BN0230M metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 1.6 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay MAI metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 8 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay N-methylnitroguanidin metabolite	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 50 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TI 435-Triazan intermediate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 50 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative
Gene mutation bacterial reverse mutation assay TI 435-CCMT-Adduct intermediate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 from 16 to 5000 µg/plate (in DMSO) in the presence and absence of a metabolic activation	Negative

SPECIAL STUDIES

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
13-week subchronic neurotoxicity dietary	Fischer 344 rats (12/sex/group) 0, 150, 1000 or 3000 ppm (equivalent to 0/0, 9.2/10.6, 60.0/71.0 or 177.0/200.1 mg/kg bw/d	**LOAEL = 3000 ppm (177.0/200.1 mg/kg bw/d)** **NOAEL = 1000 ppm (60.0/71.0 mg/kg bw/d)**	3000 ppm: ↓ bw, FC and bwg (males/females) No evidence of neurotoxicity

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Acute oral neurotoxicity gavage 0.5% methylcellulose/0.4% Tween 80	Fischer 344 rats (12/sex/group) 0, 100, 200 or 400 mg/kg bw	**LOAEL = 100 mg/kg bw** **No NOAEL established**	≥ 100 mg/kg bw: ↓ total motor and locomotor activity, ↓ arousal (males) ≥ 200 mg/kg bw: ↓ body temp. (males/females); pin point pupils, uncoordinated righting reflex, decreased activity (males); ↓ total motor and locomotor activity; ↓ arousal (females) 400 mg/kg bw: tremors and ataxia (males/females); uncoordinated gait (males); pin point pupils, impaired righting reflex, decreased activity (females)
Acute oral neurotoxicity gavage 0.5% methylcellulose/0.4% Tween 80	Fischer 344 ♂ rats (12/group) 0, 20, 40 or 60 mg/kg bw	**LOAEL was not observed** **NOAEL = 60 mg/kg bw**	No treatment-related effects noted
Developmental neurotoxicity dietary	Pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats (25/group) from GD 0 to PND 22 at doses of 0, 150, 500, or 1750 ppm (0, 12.9, 42.9, and 142 mg/kg bw/d during gestation; 0, 27.3, 90.0, and 299.0 mg/kg bw/d during lactation)	**Maternal LOAEL = 1750 ppm (142 mg/kg bw/d)** **Maternal NOAEL = 500 ppm (42.9 mg/kg bw/d)** **Offspring LOAEL = 500 ppm (42.9 mg/kg bw/d)** **Offspring NOAEL = 150 ppm (12.9 mg/kg bw/d)**	**Maternal** 142 mg/kg bw/d: ↓ bw FC and bwg (dams) **Offspring** ≥ 42.9 mg/kg bw/d: ↓ bw and bwg, ↓ motor activity and acoustic startle response (female pups) ≥ 142 mg/kg bw/d: ↓ bw and bwg, ↓ motor activity and mortality (males/females)
Acute oral gavage pharmacological studies 5% arabic gum	Male CD-1 mice, CD rats and guinea pigs tested at various doses in several studies	**LOAEL neurotoxicity (CD-1 mice) = 50 mg/kg bw** **NOAEL = 25 mg/kg bw**	**Mice** ≥ 25 mg/kg bw: ↑ tonic flexor and extensor convulsions with subthreshold shock (N/A) ≥ 50 mg/kg bw: ↓ spontaneous motor activity, ↑ tremors and deep respirations ≥ 75 mg/kg bw: ↓ intestinal transport ≥ 100 mg/kg bw/d: ↓ reactivity, grooming and muscle tone; prone position; staggering gait; mydriasis, and hypothermia ≥ 225 mg/kg bw: ↓ muscle strength and prolonged hexobarbital-induced sleeping time 400 mg/kg bw: cyanosis **Rat** ≥ 300 mg/kg bw: ↓ body temp.

STUDY	SPECIES, STRAIN AND DOSES	NOAEL AND LOAEL mg/kg bw/d	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
Acute oral neurotoxicity gavage 0.5% methylcellulose/0.4% Tween 80	Fischer 344 rats (12/sex/group) 0, 100, 200 or 400 mg/kg bw	**LOAEL = 100 mg/kg bw** **No NOAEL established**	≥ 100 mg/kg bw: ↓ total motor and locomotor activity, ↓ arousal (males) ≥ 200 mg/kg bw: ↓ body temp. (males/females); pin point pupils, uncoordinated righting reflex, decreased activity (males); ↓ total motor and locomotor activity; ↓ arousal (females) 400 mg/kg bw: tremors and ataxia (males/females); uncoordinated gait (males); pin point pupils, impaired righting reflex, decreased activity (females)
Acute oral neurotoxicity gavage 0.5% methylcellulose/0.4% Tween 80	Fischer 344 ♂ rats (12/group) 0, 20, 40 or 60 mg/kg bw	**LOAEL was not observed** **NOAEL = 60 mg/kg bw**	No treatment-related effects noted
Developmental neurotoxicity dietary	Pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats (25/group) from GD 0 to PND 22 at doses of 0, 150, 500, or 1750 ppm (0, 12.9, 42.9, and 142 mg/kg bw/d during gestation; 0, 27.3, 90.0, and 299.0 mg/kg bw/d during lactation)	**Maternal LOAEL = 1750 ppm (142 mg/kg bw/d)** **Maternal NOAEL = 500 ppm (42.9 mg/kg bw/d)** **Offspring LOAEL = 500 ppm (42.9 mg/kg bw/d)** **Offspring NOAEL = 150 ppm (12.9 mg/kg bw/d)**	**Maternal** 142 mg/kg bw/d: ↓ bw FC and bwg (dams) **Offspring** ≥ 42.9 mg/kg bw/d: ↓ bw and bwg, ↓ motor activity and acoustic startle response (female pups) ≥ 142 mg/kg bw/d: ↓ bw and bwg, ↓ motor activity and mortality (males/females)
Acute oral gavage pharmacological studies 5% arabic gum	Male CD-1 mice, CD rats and guinea pigs tested at various doses in several studies	**LOAEL neurotoxicity (CD-1 mice) = 50 mg/kg bw** **NOAEL = 25 mg/kg bw**	**Mice** ≥ 25 mg/kg bw: ↑ tonic flexor and extensor convulsions with subthreshold shock (N/A) ≥ 50 mg/kg bw: ↓ spontaneous motor activity, ↑ tremors and deep respirations ≥ 75 mg/kg bw: ↓ intestinal transport ≥ 100 mg/kg bw/d: ↓ reactivity, grooming and muscle tone; prone position; staggering gait; mydriasis, and hypothermia ≥ 225 mg/kg bw: ↓ muscle strength and prolonged hexobarbital-induced sleeping time 400 mg/kg bw: cyanosis **Rat** ≥ 300 mg/kg bw: ↓ body temp.

Compound-induced mortality:
The compound produced much greater toxicity in mice than in rats in acute lethality studies (LD_{50} values of 389 mg/kg bw and greater than 5000 mg/kg bw in mice and rats respectively). The end-use product Poncho 600 had an acute $LD_{50} > 500$ and < 1000 mg/kg bw in rats, demonstrating greater toxicity for the end-use product than for the TGAI. Mortalities (1 male and 1 female) occurred in the 28 day dog study at doses of 36.9 and 53.5 mg/kg bw/d. Mortalities occurred in the 28 day mouse study as well (2/6 males at 383 mg/kg bw/d, with 10/12 animals dying in the first 2 weeks following dosing at 683/619 mg/kg bw/d [male/female]). Decreased survival occurred in the chronic mouse study at doses of 322.3 mg/kg bw/d, while pregnant rabbits died (2/23 and 3/23) following dosing at 75 or 100 mg/kg bw/d, respectively. Mortalities occurred between gestation days 17 and 27.

Recommended ARfD: The ARfD for women age 13 + is 0.25 mg/kg bw, based on the NOAEL of 25 mg/kg bw/d established in the rabbit developmental toxicity study (protect against missing lobe of the lung in developing young). For the general population, the ARfD is based on a similar NOAEL of 25 mg/kg bw in the acute oral pharmacology study in the mouse (early onset of clinical signs of toxicity) with a 100-fold uncertainty factor.
MOE for other critical endpoint(s): 100

Recommended ADI: The ADI is 0.0327 mg/kg bw/d, based on the NOAEL of 9.8 established in the two-generation reproduction study, with a 300-fold uncertainty factor.
MOE for other critical endpoint(s): 300

Appendix IV Residues

Table 1 Integrated food residue chemistry summary table

DIRECTIONS FOR THE SEED TREATMENT USE OF CLOTHIANIDIN			
Crop	**Pest**	**Approved rates**	
		EP	**a.i.**
Canola, rapeseed	Flea beetle	250 or 666 mL/ 100 kg seed	150 or 400 g/ 100 kg seed
Corn (sweet, field and pop)	Corn rootworm (including northern and western)	166.7 mL/ 80,000 seed	1.25 mg/ kernel
	Corn flea beetle, black cutworm, seedcorn maggot, wireworm	33.3–66.6 mL/80 000 seed	0.25–0.5 mg/kernel
	White grub (larvae of European chafer, May/June beetle and Japanese beetle)	33.3 mL/80 000 seed	0.25 mg/kernel

PHYSICOCHEMICAL PROPERTIES	
Water solubility at 20°C	0.327 g/L
Solvent solubility at 25°C (g/L)	acetone (15.2), dichloromethane (1.32), ethyl acetate (2.03), heptane (<0.00104), methanol (6.26), octanol (0.938), xylene (0.0128)
n-Octanol–water partition coefficient (Log K_{ow})	0.7
Dissociation constant (pK_a) at 20°C	11.09
Vapour pressure at 25°C	1.3×10^{-10} Pa
Relative density at 20°C	1.59 g/cm^3
Melting point	176.8°C
pH	6.24 (1% solution/suspension) at 23°C
UV–visible absorption spectrum	Max 265.5 nm in acidic and neutral solution; max 246 nm in basic solution

<table>
<tr><th colspan="3">ANALYTICAL METHODOLOGY</th></tr>
<tr><th>Parameters</th><th>Plant matrices</th><th>Animal matrices</th></tr>
<tr><td>Method ID</td><td>Method 00552 + M001</td><td>Method 00624</td></tr>
<tr><td>Type</td><td>Data gathering and enforcement</td><td>Enforcement</td></tr>
<tr><td>Analytes</td><td>TI-435</td><td>TI-435, TZG, TZU and ATMG-Pyr</td></tr>
<tr><td>Instrumentation</td><td colspan="2">HPLC using a C18 column, a gradient mobile phase of acidic water and acetonitrile and MS/MS identification and quantitation.</td></tr>
<tr><td>LOQ</td><td>0.01 ppm for corn and canola matrices</td><td>0.01 ppm for each analyte in milk and 0.02 ppm for each analyte in animal tissues</td></tr>
<tr><td>Standard</td><td colspan="2">External bracketing standards and internal d_3-TI-435 standard.</td></tr>
<tr><td>ILV</td><td>Acceptable validation of Method 00552-M001 was completed with corn grain.</td><td>Acceptable validation of Method 00624 was completed with milk, muscle, fat and liver.</td></tr>
<tr><td>Extraction</td><td>Residues are extracted with acetonitrile/water, filtered, and the filtrate is concentrated for clean-up through a ChemElut column eluted with cyclohexane/ethyl acetate.</td><td>Residues in tissues are extracted with acetonitrile/water, and the extract is concentrated for clean-up through a Bond Elut™ ENV column eluted with acidic water/methanol. Fat samples are extracted with acetonitrile/water and hexane, and the extract is partitioned between acetonitrile and hexane; the acetonitrile/water phase is concentrated for clean-up through an ENV column. Milk samples are simply diluted with water and applied to an ENV column for clean-up.</td></tr>
<tr><td>Radiovalidation</td><td>Corn forage (81%); corn stover (74%); corn grain (61%) and apples (85%).</td><td>Bioincurred residues of TI-435, TZG, TZU and ATMG-Pyr were adequately recovered in milk, muscle, fat, liver, with the exception of ATGM-Pyruvate in fat (45.6%).</td></tr>
<tr><td>Multiresidue method</td><td colspan="2">TI-435 and the metabolites TZG, MNG, TZNG, TZU and ATMG-pyruvate were not adequately recovered using any of the prescribed protocols in the USFDA PAM, Vol. 1, Multiresidue Methods (1994).</td></tr>
</table>

Appendix IV Residues

Table 1 Integrated food residue chemistry summary table

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PHYSICOCHEMICAL PROPERTIES	
Water solubility at 20°C	0.327 g/L
Solvent solubility at 25°C (g/L)	acetone (15.2), dichloromethane (1.32), ethyl acetate (2.03), heptane (<0.00104), methanol (6.26), octanol (0.938), xylene (0.0128)
n-Octanol–water partition coefficient (Log K_{ow})	0.7
Dissociation constant (pK_a) at 20°C	11.09
Vapour pressure at 25°C	1.3×10^{-10} Pa
Relative density at 20°C	1.59 g/cm^3
Melting point	176.8°C
pH	6.24 (1% solution/suspension) at 23°C
UV–visible absorption spectrum	Max 265.5 nm in acidic and neutral solution; max 246 nm in basic solution

ANALYTICAL METHODOLOGY		
Parameters	**Plant matrices**	**Animal matrices**
Method ID	Method 00552 + M001	Method 00624
Type	Data gathering and enforcement	Enforcement
Analytes	TI-435	TI-435, TZG, TZU and ATMG-Pyr
Instrumentation	HPLC using a C18 column, a gradient mobile phase of acidic water and acetonitrile and MS/MS identification and quantitation.	
LOQ	0.01 ppm for corn and canola matrices	0.01 ppm for each analyte in milk and 0.02 ppm for each analyte in animal tissues
Standard	External bracketing standards and internal d_3-TI-435 standard.	
ILV	Acceptable validation of Method 00552-M001 was completed with corn grain.	Acceptable validation of Method 00624 was completed with milk, muscle, fat and liver.
Extraction	Residues are extracted with acetonitrile/water, filtered, and the filtrate is concentrated for clean-up through a ChemElut column eluted with cyclohexane/ethyl acetate.	Residues in tissues are extracted with acetonitrile/water, and the extract is concentrated for clean-up through a Bond Elut™ ENV column eluted with acidic water/methanol. Fat samples are extracted with acetonitrile/water and hexane, and the extract is partitioned between acetonitrile and hexane; the acetonitrile/water phase is concentrated for clean-up through an ENV column. Milk samples are simply diluted with water and applied to an ENV column for clean-up.
Radiovalidation	Corn forage (81%); corn stover (74%); corn grain (61%) and apples (85%).	Bioincurred residues of TI-435, TZG, TZU and ATMG-Pyr were adequately recovered in milk, muscle, fat, liver, with the exception of ATGM-Pyruvate in fat (45.6%).
Multiresidue method	TI-435 and the metabolites TZG, MNG, TZNG, TZU and ATMG-pyruvate were not adequately recovered using any of the prescribed protocols in the USFDA PAM, Vol. 1, Multiresidue Methods (1994).	

NATURE OF THE RESIDUE IN PLANTS

Crop	Corn		Sugar beet	Tomato		Apple
Radiolabel	nitroimino	thiazolyl	nitroimino	nitroimino		nitroimino
Test site	quasi-greenhouse	greenhouse	field	greenhouse		greenhouse
Treatment	seed	seed	seed	foliar	soil	foliar
Rate	1.06 mg a.i./seed	2.52 mg a.i./seed	190 g a.i./ha	2 × 158 g a.i./ha	15 mg a.i./plant	2 × 202 g a.i./ha
Seasonal Rate	1.06 mg a.i./seed	2.52 mg a.i./seed	190 g a.i./ha	316 g a.i./ha	15 mg a.i./plant	404 g a.i./ha
EP	WS 70	WS 70	WS 70	SC 200	GR 0.5	SC 200
PHI (days)	145	160	144	3	97	14
Major metabolites (> 10% of the TRRs)	TI-435, MG		TI-435, MG, TMG	TI-435, MNG		TI-435, TZMU
Minor metabolites	TZNG, TZMU, TMG, TZU, MG, MNG, NTG, CTCA		TZNG, TZMU, TMG, TZU, MG, MNG, NTG	TZNG		TZNG, MG, MNG, NTG, THMN-Glc, TMG, TZU
Residue of concern	The parent only, TI-435, is the residue of concern. Methyl guanidine (MG) was discounted because it is naturally occurring in mammals and plants. TMG and TZMU were also excluded as unlikely to contribute to toxicological effects seen in rat study.					

CONFINED ROTATIONAL CROP STUDY—TURNIP, SWISS CHARD AND WHEAT

Formulation used for trial	SC 200 (soluble concentrate formulation)
Application rate and timing	328 g a.i./ha (0.293 lb a.i./acre). Applied directly to bare soil prior to planting rotational crops.

Succeeding crops	Major metabolites (> 10% TRRs)	Minor metabolites
WHEAT—29 D PBI 314 D PBI	TI-435, TZNG, MNG TI-435, TZNG, MNG, MG	TZNG, TZMU, TMG, TZU, MG, MNG, NTG TZNG, TZMU, TMG, TZU, MG, MNG, NTG
SWISS CHARD—29 D PBI 314 D PBI	TI-435, TZNG, MNG TI-435, NTG, MNG	TZMU, TMG, TZU, MG, NTG TZNG, TZMU, TMG, TZU, MG
TURNIP—29 D PBI 314 D PBI	TI-435, MNG TI-435, MNG	TZNG, TZMU, TMG, TZU, MG, MNG, NTG TZNG, TZMU, TMG, TZU, MG, MNG, NTG

Residue of concern	Parent, TI-435, only for maximum residue limit expression. However TZNG and MNG could not be discounted as being significantly less toxic than the parent, and will be included in the risk assessment.

NATURE OF THE RESIDUE IN LIVESTOCK

Species	Radiolabel	Dose level	Sacrifice
Goat (*Bunte Deutsche Edelziege*)	[Nitroimino-^{14}C] TI-435 in 0.5% aqueous tragacanth suspension; 105.7 μCi/mg single label	9.8 mg/kg bw/d for three consecutive days, equivalent to 200 mg/kg in feed	53 h after first administration
Hen (White Leghorn laying hens)	[Nitroimino-^{14}C] in 0.5% aqueous tragacanth suspension; 102.2 μCi/mg single label	10.4 mg/kg bw/d for three consecutive days, equivalent to 140 mg/kg in feed	53 h after first administration

Matrices	Major metabolites (> 10% TRRs)	Minor metabolites
Hen muscle	ATG-Ac	TI-435, TZNG, TMG, TZU, MNG, NTG, TZG, ATMT, TMT, urea
Hen fat	ATG-Ac, TZNG	TI-435, ATG-Pyr
Hen liver	TZNG, TZG	TI-435, TMG, TZU, MNG, NTG, urea
Hen eggs	TI-435, TZNG	TZU, MNG, NTG, urea
Goat muscle	TI-435, TZU	TZNG, TZMU, TMG, MG, MNG, NTG, TZG, ATMG-Pyr, urea
Goat liver	TMG-adducts	MG, urea, NTG, TZG, TMG, TZU, TZMU, ATMG-Pyr, TZNG
Goat kidney	TZMU, TZU, TZG, ATMG-Pyr	TZNG, TZMU, TMG, MNG, NTG, urea
Goat fat	TI-435, TZMU, TZU	TZNG, TZMU, TMG, MNG, NTG, TZG, ATMG-Pyr, urea
Goat milk	TI-435, TZNG, TZU	TZMU, TMG, MNG, NTG, TMHG, urea
Residue of concern	TI-435. TZMU excluded since structurally it does not contain the nitro group, which is believed to contribute to neurotoxicity. TZU, TZG, TZNG, ATMG-pyruvate, ATG-acetate included in risk assessment because no toxicological information on these.	

STORAGE STABILITY

Residues of clothianidin (not the metabolites) were stable for at least 24 months in sugar beet, corn and canola. No corrections to residue values due to in-storage dissipation are necessary.

CROP FIELD TRIALS—CORN AND CANOLA AS SEED TREATMENT

Crop field trials in field, pop and sweet corn were conducted in representative zones (1, 2, 3, 5, 5B, 6, 7A, 10, 11, 12). Canola trials conducted in representative zones (2, 5, 7, 11, 14).

NATURE OF THE RESIDUE IN PLANTS

Crop	Corn		Sugar beet	Tomato		Apple
Radiolabel	nitroimino	thiazolyl	nitroimino	nitroimino		nitroimino
Test site	quasi-greenhouse	greenhouse	field	greenhouse		greenhouse
Treatment	seed	seed	seed	foliar	soil	foliar
Rate	1.06 mg a.i./seed	2.52 mg a.i./seed	190 g a.i./ha	2 × 158 g a.i./ha	15 mg a.i./plant	2 × 202 g a.i./ha
Seasonal Rate	1.06 mg a.i./seed	2.52 mg a.i./seed	190 g a.i./ha	316 g a.i./ha	15 mg a.i./plant	404 g a.i./ha
EP	WS 70	WS 70	WS 70	SC 200	GR 0.5	SC 200
PHI (days)	145	160	144	3	97	14
Major metabolites (> 10% of the TRRs)	TI-435, MG		TI-435, MG, TMG	TI-435, MNG		TI-435, TZMU
Minor metabolites	TZNG, TZMU, TMG, TZU, MG, MNG, NTG, CTCA		TZNG, TZMU, TMG, TZU, MG, MNG, NTG	TZNG		TZNG, MG, MNG, NTG, THMN-Glc, TMG, TZU
Residue of concern	The parent only, TI-435, is the residue of concern. Methyl guanidine (MG) was discounted because it is naturally occurring in mammals and plants. TMG and TZMU were also excluded as unlikely to contribute to toxicological effects seen in rat study.					

CONFINED ROTATIONAL CROP STUDY—TURNIP, SWISS CHARD AND WHEAT

Formulation used for trial	SC 200 (soluble concentrate formulation)
Application rate and timing	328 g a.i./ha (0.293 lb a.i./acre). Applied directly to bare soil prior to planting rotational crops.

Succeeding crops	Major metabolites (> 10% TRRs)	Minor metabolites
WHEAT—29 D PBI 314 D PBI	TI-435, TZNG, MNG TI-435, TZNG, MNG, MG	TZNG, TZMU, TMG, TZU, MG, MNG, NTG TZNG, TZMU, TMG, TZU, MG, MNG, NTG
SWISS CHARD—29 D PBI 314 D PBI	TI-435, TZNG, MNG TI-435, NTG, MNG	TZMU, TMG, TZU, MG, NTG TZNG, TZMU, TMG, TZU, MG
TURNIP—29 D PBI 314 D PBI	TI-435, MNG TI-435, MNG	TZNG, TZMU, TMG, TZU, MG, MNG, NTG TZNG, TZMU, TMG, TZU, MG, MNG, NTG

Residue of concern	Parent, TI-435, only for maximum residue limit expression. However TZNG and MNG could not be discounted as being significantly less toxic than the parent, and will be included in the risk assessment.

NATURE OF THE RESIDUE IN LIVESTOCK

Species	Radiolabel	Dose level	Sacrifice
Goat (*Bunte Deutsche Edelziege*)	[Nitroimino-^{14}C] TI-435 in 0.5% aqueous tragacanth suspension; 105.7 µCi/mg single label	9.8 mg/kg bw/d for three consecutive days, equivalent to 200 mg/kg in feed	53 h after first administration
Hen (White Leghorn laying hens)	[Nitroimino-^{14}C] in 0.5% aqueous tragacanth suspension; 102.2 µCi/mg single label	10.4 mg/kg bw/d for three consecutive days, equivalent to 140 mg/kg in feed	53 h after first administration

Matrices	Major metabolites (> 10% TRRs)	Minor metabolites
Hen muscle	ATG-Ac	TI-435, TZNG, TMG, TZU, MNG, NTG, TZG, ATMT, TMT, urea
Hen fat	ATG-Ac, TZNG	TI-435, ATG-Pyr
Hen liver	TZNG, TZG	TI-435, TMG, TZU, MNG, NTG, urea
Hen eggs	TI-435, TZNG	TZU, MNG, NTG, urea
Goat muscle	TI-435, TZU	TZNG, TZMU, TMG, MG, MNG, NTG, TZG, ATMG-Pyr, urea
Goat liver	TMG-adducts	MG, urea, NTG, TZG, TMG, TZU, TZMU, ATMG-Pyr, TZNG
Goat kidney	TZMU, TZU, TZG, ATMG-Pyr	TZNG, TZMU, TMG, MNG, NTG, urea
Goat fat	TI-435, TZMU, TZU	TZNG, TZMU, TMG, MNG, NTG, TZG, ATMG-Pyr, urea
Goat milk	TI-435, TZNG, TZU	TZMU, TMG, MNG, NTG, TMHG, urea
Residue of concern	TI-435. TZMU excluded since structurally it does not contain the nitro group, which is believed to contribute to neurotoxicity. TZU, TZG, TZNG, ATMG-pyruvate, ATG-acetate included in risk assessment because no toxicological information on these.	

STORAGE STABILITY

Residues of clothianidin (not the metabolites) were stable for at least 24 months in sugar beet, corn and canola. No corrections to residue values due to in-storage dissipation are necessary.

CROP FIELD TRIALS—CORN AND CANOLA AS SEED TREATMENT

Crop field trials in field, pop and sweet corn were conducted in representative zones (1, 2, 3, 5, 5B, 6, 7A, 10, 11, 12). Canola trials conducted in representative zones (2, 5, 7, 11, 14).

Commodity	Total Applic. Rate g a.i./ha	PHI (days)	Analyte	Residue Levels (ppm)				
				Min.	Max.	HAFT	Mean	SDEV
Forage w/ears	121.8–187.3	72–113	TI-435	< 0.002	0.061	0.056	0.01	0.011
Ears	121.8–187.3	75–115	TI-435	< 0.001	0.01	0.01	0	0.01
Forage	121.8–187.3	83–134	TI-435	< 0.002	0.037	0.035	0.01	0.01
Fodder	121.8–187.3	119–159	TI-435	< 0.002	0.052	0.045	0.01	0.013
Grain	121.8–187.3	119–159	TI-435	< 0.001	0.01	0.01	0	0
Canola seeds	39.9–43.3	92–136	TI-435	< 0.002	0	0	0	0

MAXIMUM RESIDUE LIMITS

corn, field, grain; corn, pop, grain; corn, sweet, kernel plus cob with husk removed; canola, seed	0.01 ppm

FIELD ACCUMULATION IN ROTATIONAL CROPS—MUSTARD GREENS, TURNIP, WHEAT

The application rate to the primary crop (corn) was 2 mg a.i./seed. Residues of clothianidin and TZNG were reported. Only turnip roots, wheat straw and wheat grain had residues less than 0.01 ppm (LOQ) for each clothianidin and TZNG at the 30-day PBI. Clothianidin residues increased over the various PBIs for mustard greens, turnip tops, wheat forage and wheat hay. At 357–366 days PBI, residues of clothianidin in all succeeding crops were less than LOQ. Therefore, a PBI of 30 days can only be supported for cereal grains, grasses, non-grass animal feeds and soybeans/dried beans. Leafy, root and tuber vegetables must have a 12-month PBI. Corn and canola may be replanted immediately.

PROCESSED FOOD AND FEED

Residues of clothianidin were less than 0.01 ppm (LOQ) in both canola seed and corn grain. Therefore, no further analyses of the processed commodities were conducted. No concentration factor considered for petitioned uses.

LIVESTOCK FEEDING

Clothianidin administered orally to dairy cattle at 0.26, 0.8 and 2.56 mg/kg feed. Residues of TI-435, TZG, TZU and ATMG-pyruvate were each less than 0.01 ppm (LOQ) in milk. Residues of TI-435, TZG, TZU and ATMG-pyruvate were each less than 0.02 ppm (LOQ) in muscle, kidney, liver and fat tissues. The maximum theoretical dietary burden (MTDB) was estimated taking into account the contribution from residues of clothianidin in livestock feed items, as a result of seed treatment using clothianidin and thiamethoxam. Clothianidin is the residue of concern for thiamethoxam. The anticipated dietary burden is 0.37 ppm for dairy cattle and 0.28 ppm in beef cattle. The anticipated residue of clothianidin is 0.0017 ppm in milk and 0.0029 ppm in tissues, which represent a conservative estimate.

Table 2 Overview of plant/animal metabolism studies and risk assessment

PLANT STUDIES	
CROPS (N=4)	**Clothianidin**
	tomato, apple, corn, sugar beet
ROC FOR MONITORING AND MAXIMUM RESIDUE LIMIT	Parent only (TI-435)
ROC FOR RISK ASSESSMENT	Plants: parent only (TI-435) Rotational crops: parent, TZNG, MNG
METABOLIC PROFILE IN DIVERSE CROPS	Future new uses on root crops and/or leafy vegetables will require field trials to analyze TMG or the submission of more metabolism data, preferably, side-by-side thiazol- and nitroimino radiolabel studies.
ANIMAL STUDIES	
ANIMALS (N=2)	Goat and hen
ROC FOR MONITORING AND MAXIMUM RESIDUE LIMIT	Parent only
ROC FOR RISK ASSESSMENT	cattle: clothianidin, TZU, TZG, TZNG, ATMG–pyruvate poultry: clothianidin, TZU, TZG, TZNG, ATG-acetate
METABOLIC PROFILE IN ANIMALS	Some unique metabolites were found in hen, goat and rat due to the fact that only the nitroimino label was used in livestock studies.
FAT SOLUBLE RESIDUE	No

Commodity	Total Applic. Rate g a.i./ha	PHI (days)	Analyte	Residue Levels (ppm)				
				Min.	Max.	HAFT	Mean	SDEV
Forage w/ears	121.8–187.3	72–113	TI-435	< 0.002	0.061	0.056	0.01	0.011
Ears	121.8–187.3	75–115	TI-435	< 0.001	0.01	0.01	0	0.01
Forage	121.8–187.3	83–134	TI-435	< 0.002	0.037	0.035	0.01	0.01
Fodder	121.8–187.3	119–159	TI-435	< 0.002	0.052	0.045	0.01	0.013
Grain	121.8–187.3	119–159	TI-435	< 0.001	0.01	0.01	0	0
Canola seeds	39.9–43.3	92–136	TI-435	< 0.002	0	0	0	0

MAXIMUM RESIDUE LIMITS

corn, field, grain; corn, pop, grain; corn, sweet, kernel plus cob with husk removed; canola, seed	0.01 ppm

FIELD ACCUMULATION IN ROTATIONAL CROPS—MUSTARD GREENS, TURNIP, WHEAT

The application rate to the primary crop (corn) was 2 mg a.i./seed. Residues of clothianidin and TZNG were reported. Only turnip roots, wheat straw and wheat grain had residues less than 0.01 ppm (LOQ) for each clothianidin and TZNG at the 30-day PBI. Clothianidin residues increased over the various PBIs for mustard greens, turnip tops, wheat forage and wheat hay. At 357–366 days PBI, residues of clothianidin in all succeeding crops were less than LOQ. Therefore, a PBI of 30 days can only be supported for cereal grains, grasses, non-grass animal feeds and soybeans/dried beans. Leafy, root and tuber vegetables must have a 12-month PBI. Corn and canola may be replanted immediately.

PROCESSED FOOD AND FEED

Residues of clothianidin were less than 0.01 ppm (LOQ) in both canola seed and corn grain. Therefore, no further analyses of the processed commodities were conducted. No concentration factor considered for petitioned uses.

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ROC FOR RISK ASSESSMENT	*Plants: parent only (TI-435)* Rotational crops: parent, TZNG, MNG
METABOLIC PROFILE IN DIVERSE CROPS	Future new uses on root crops and/or leafy vegetables will require field trials to analyze TMG or the submission of more metabolism data, preferably, side-by-side thiazol- and nitroimino radiolabel studies.
ANIMAL STUDIES	
ANIMALS (N=2)	Goat and hen
ROC FOR MONITORING AND MAXIMUM RESIDUE LIMIT	Parent only
ROC FOR RISK ASSESSMENT	cattle: clothianidin, TZU, TZG, TZNG, ATMG-pyruvate poultry: clothianidin, TZU, TZG, TZNG, ATG-acetate
METABOLIC PROFILE IN ANIMALS	Some unique metabolites were found in hen, goat and rat due to the fact that only the nitroimino label was used in livestock studies.
FAT SOLUBLE RESIDUE	No

DIETARY RISK FROM FOOD AND WATER			
Chronic non-cancer dietary risk **ADI = 0.033 mg/kg bw** **EEC = 13.2 μg a.i./L (Tier II)**	**POPULATION**	**ESTIMATED RISK (% of ADI)**	
		Food (MRLs)	**Food + EEC**
	All infants < 1 yr old	1.2	4
	Children 1 to 2 yrs	2.3	3.6
	Children 3 to 5 yrs	1.7	2.9
	Children 6 to 12 yrs	1	1.8
	Youths 13 to 19 yrs	0.5	1.2
	Adults 20 to 49 yrs	0.4	1.2
	Adults 50+ yrs	0.4	1.2
	Females 13 to 49 yrs	0.4	1.2
	Total population	0.6	1.4
Acute dietary exposure analysis, deterministic, 95th percentile	**POPULATION**	**ESTIMATED RISK (% of ARfD)**	
		Food (MRLs)	**Food + EEC**
ARfD = 0.25 mg/kg bw	**Females 13 +**	0.15	0.3
ARfD = 0.25 mg/kg bw	**Total population**	0.24	0.46
Cancer risk assessment **$Q_1^* = 2.33 \times 10^{-3}$**	**Population**	**Food (STMRs)**	**Food + EEC (STMRs)**
	All infants < 1 yr old	2.99×10^{-7}	2.40×10^{-6}
	Children 1 to 2 yrs	6.50×10^{-7}	1.60×10^{-6}
	Children 3 to 5 yrs	4.66×10^{-7}	1.36×10^{-6}
	Children 6 to 12 yrs	2.78×10^{-7}	8.92×10^{-7}
	Youths 13 to 19 yrs	1.42×10^{-7}	6.05×10^{-7}
	Adults 20 to 49 yrs	1.01×10^{-7}	6.99×10^{-7}
	Adults 50+ yrs	9.34×10^{-8}	7.22×10^{-7}
	Females 13 to 49 yrs	1.01×10^{-7}	6.96×10^{-7}
	Total population	1.59×10^{-7}	7.99×10^{-7}

DIETARY RISK FROM FOOD AND WATER			
Chronic non-cancer dietary risk **ADI = 0.033 mg/kg bw** **EEC = 13.2 µg a.i./L (Tier II)**	**POPULATION**	**ESTIMATED RISK (% of ADI)**	
		Food (MRLs)	**Food + EEC**
	All infants < 1 yr old	1.2	4
	Children 1 to 2 yrs	2.3	3.6
	Children 3 to 5 yrs	1.7	2.9
	Children 6 to 12 yrs	1	1.8
	Youths 13 to 19 yrs	0.5	1.2
	Adults 20 to 49 yrs	0.4	1.2
	Adults 50+ yrs	0.4	1.2
	Females 13 to 49 yrs	0.4	1.2
	Total population	0.6	1.4
Acute dietary exposure analysis, deterministic, 95th percentile	**POPULATION**	**ESTIMATED RISK (% of ARfD)**	
		Food (MRLs)	**Food + EEC**
ARfD = 0.25 mg/kg bw	**Females 13 +**	0.15	0.3
ARfD = 0.25 mg/kg bw	**Total population**	0.24	0.46
Cancer risk assessment **$Q_1^* = 2.33 \times 10^{-3}$**	**Population**	**Food (STMRs)**	**Food + EEC (STMRs)**
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	Children 1 to 2 yrs	6.50×10^{-7}	1.60×10^{-6}
	Children 3 to 5 yrs	4.66×10^{-7}	1.36×10^{-6}
	Children 6 to 12 yrs	2.78×10^{-7}	8.92×10^{-7}
	Youths 13 to 19 yrs	1.42×10^{-7}	6.05×10^{-7}
	Adults 20 to 49 yrs	1.01×10^{-7}	6.99×10^{-7}
	Adults 50+ yrs	9.34×10^{-8}	7.22×10^{-7}
	Females 13 to 49 yrs	1.01×10^{-7}	6.96×10^{-7}
	Total population	1.59×10^{-7}	7.99×10^{-7}

Appendix V Environmental assessment

Table 1 Physical and chemical properties of the active ingredient relevant to the environment

Property	Value	Comments
Water solubility at 20°C	327 mg a.i/L	Very soluble in water. One of the indicators of high potential to leach
Vapour pressure	1.3×10^{-10} Pa at 25°C 3.8×10^{-11} Pa at 20°C (extrapolated at 20°C)	Non-volatile from water and moist soil surface
Henry's Law constant at 25°C	$1/H = 2.5 \times 10^{13}$ $K = 9.8 \times 10^{-16}$ atm m^3/mole	
K_{ow} log K_{ow}	5 0.7 at 25°C	Low potential for bioaccumulation
pK_a	11.09 at 20°C	Under acidic and neutral conditions, clothianidin will be in the undissociated form.
UV–visible absorption	Maximum of 265.5 nm in acidic and neutral solution, maximum of 246.0 nm in basic solution	Minimal phototransformation is expected in the natural environment.

Table 2 Summary of results of terrestrial field dissipation studies

Study	Location (Ecoregion)	Persistence	Carry-over		Leaching	Transformation products	
		DT_{50}* (days)	DT_{90}* (days)	Residues (%)**	Maximum depth	Major	Minor
Canada	Branchton, Ontario (8.1)	365 persistent	1279	80	30 cm	none	TZNG, MNG, TZMU, TMG
	Saskatoon, Saskatchewan (9.3)	ND persistent§	ND	91	30 cm	none	MNG, TZNG, TMG

Study	Location (Ecoregion)	Persistence	Carry-over		Leaching	Transformation products	
		DT_{50}* (days)	DT_{90}* (days)	Residues (%)**	Maximum depth	Major	Minor
United States	Northwood, North Dakota (9.2)	1386 persistent	4606	134	45 cm	none	TZNG, MNG, TZMU
	New Holland, Ohio (8.2)	277 persistent	980	52	60 cm	none	TZNG, MNG, TZMU
	Arena, Wisconsin (8.2)	315 persistent	1355	89	60 cm	none	TZNG, MNG, TZMU

* total residues in all the soil depths

** % of 0-day concentration carried to the following crop season (end of 4–6 month period for canola and corn)

§ ND: not determined due to very limited dissipation

Table 3 Fate and behaviour in the terrestrial environment

Property	Test substance	Value	Comments
Abiotic transformation			
Hydrolysis	clothianidin	did not hydrolyze at pH 5, pH 7 and pH 9	not an important route of transformation in the environment
Phototransformation in air	clothianidin	not required—not volatile	
Biotransformation			
Biotransformation in aerobic soil	clothianidin	495–990 d	persistent in soil major transformation products are TZNG and MNG (10–11% AR) minor transformation products are TZMU, NTG, CO2 (17%)
Mobility			
Adsorption/ desorption in soil	clothianidin	adsorption $K_d = 0.5–4.14$ adsorption $K_{oc} = 84–345$	moderate to high mobility
	MNG	adsorption $K_d = 0.02–0.4$ adsorption $K_{oc} = 5.2–34.3$	very high mobility
	TZNG	adsorption $K_d = 0.6–4.7$ adsorption $K_{oc} = 205–433$	moderate mobility

Appendix V Environmental assessment

Table 1 Physical and chemical properties of the active ingredient relevant to the environment

Property	Value	Comments
Water solubility at 20°C	327 mg a.i/L	Very soluble in water. One of the indicators of high potential to leach
Vapour pressure	1.3×10^{-10} Pa at 25°C 3.8×10^{-11} Pa at 20°C (extrapolated at 20°C)	Non-volatile from water and moist soil surface
Henry's Law constant at 25°C	$1/H = 2.5 \times 10^{13}$ $K = 9.8 \times 10^{-16}$ atm m^3/mole	
K_{ow} log K_{ow}	5 0.7 at 25°C	Low potential for bioaccumulation
pK_a	11.09 at 20°C	Under acidic and neutral conditions, clothianidin will be in the undissociated form.
UV–visible absorption	Maximum of 265.5 nm in acidic and neutral solution, maximum of 246.0 nm in basic solution	Minimal phototransformation is expected in the natural environment.

Table 2 Summary of results of terrestrial field dissipation studies

Study	Location (Ecoregion)	Persistence	Carry-over		Leaching	Transformation products	
		DT_{50}* (days)	DT_{90}* (days)	Residues (%)**	Maximum depth	Major	Minor
Canada	Branchton, Ontario (8.1)	365 persistent	1279	80	30 cm	none	TZNG, MNG, TZMU, TMG
	Saskatoon, Saskatchewan (9.3)	ND persistent§	ND	91	30 cm	none	MNG, TZNG, TMG

Study	Location (Ecoregion)	Persistence	Carry-over		Leaching	Transformation products	
		DT_{50}* (days)	DT_{90}* (days)	Residues (%)**	Maximum depth	Major	Minor
United States	Northwood, North Dakota (9.2)	1386 persistent	4606	134	45 cm	none	TZNG, MNG, TZMU
	New Holland, Ohio (8.2)	277 persistent	980	52	60 cm	none	TZNG, MNG, TZMU
	Arena, Wisconsin (8.2)	315 persistent	1355	89	60 cm	none	TZNG, MNG, TZMU

* total residues in all the soil depths

** % of 0-day concentration carried to the following crop season (end of 4-6 month period for canola and corn)

\$ ND: not determined due to very limited dissipation

Table 3 Fate and behaviour in the terrestrial environment

Property	Test substance	Value	Comments
Abiotic transformation			
Hydrolysis	clothianidin	did not hydrolyze at pH 5, pH 7 and pH 9	not an important route of transformation in the environment
Phototransformation in air	clothianidin	not required—not volatile	
Biotransformation			
Biotransformation in aerobic soil	clothianidin	495–990 d	persistent in soil major transformation products are TZNG and MNG (10–11% AR) minor transformation products are TZMU, NTG, CO2 (17%)
Mobility			
Adsorption/ desorption in soil	clothianidin	adsorption $K_d = 0.5–4.14$ adsorption $K_{oc} = 84–345$	moderate to high mobility
	MNG	adsorption $K_d = 0.02–0.4$ adsorption $K_{oc} = 5.2–34.3$	very high mobility
	TZNG	adsorption $K_d = 0.6–4.7$ adsorption $K_{oc} = 205–433$	moderate mobility

Property	Test substance	Value	Comments
	TZMU	adsorption $K_d = 0.13–0.65$ adsorption $K_{oc} = 46–96$	very high to high mobility
	TMG	adsorption $K_d = 2.4–39$ adsorption $K_{oc} = 525–6159$	low mobility—immobile
Soil leaching	not required—addressed by the adsorption/desorption study		
Volatilization	not required—not volatile		
Field studies			
Field dissipation (relevant Canadian studies)	clothianidin	$DT_{50} = 385–1386$ d $DT_{90} = 1279–4606$ d % carry-over = > 80% after 4–6 months	persistent under field conditions no major transformation products formed minor transformation products are TZNG, MNG, TZMU and TMG
Field leaching		No residues detected below 0–45 cm soil depth.	low potential for leaching

Table 4 Estimated environmental concentrations (Level 1) of clothianidin in drinking water sources

Groundwater (µg a.i./L)	Reservoir (µg a.i./L)		Dugout (µg a.i./L)	
Annual average concentration[a]	Acute[b]	Chronic[c]	Acute[b]	Chronic[c]
37.1	3	0.28	1.7	0.38

[a] Maximum yearly average for a 20-year simulation

[b] 90th percentile of yearly peaks

[c] 90th percentile of yearly averages

Table 5 The estimated environmental concentrations (Level 2) of clothianidin in drinking water sources

	Groundwater (µg a.i./L)	
	90th Percentile	50th Percentile
Ontario	23.7103	22.1301
Quebec	16.48417	13.22874

Table 6 Summary of effects of clothianidin on terrestrial organisms

Group	Organism	Study	NOEL/NOEC	LD_{50}/LC_{50}/EC_{25}	Degree of toxicity
Birds	bobwhite quail	acute oral	500 mg/kg bw	> 2000 mg/kg bw	practically non-toxic
	Japanese quail	acute oral	12.5 mg/kg bw	423 mg/kg bw	moderately toxic
	bobwhite quail	acute dietary	309 mg/kg diet	5230 mg/kg diet	practically non-toxic
	mallard duck	acute dietary	646 mg/kg diet	> 5040 mg/kg diet	practically non-toxic
	bobwhite quail	reproductive	205 mg/kg diet	—	—
	mallard duck	reproductive	205 mg/kg diet	—	—
Mammals	rat	acute oral	—	> 5000 mg/kg bw	low
	mouse	acute oral	—	389 mg/kg bw	high
	rat	dermal	—	> 2000 mg/kg bw	low
	rat	inhalation	—	> 5.53 mg/L	low
	rat	reproductive	31.2 mg/kg bw/d for parent	—	high
			9.8 mg/kg bw/d for offspring	—	high
	Beagle dog	chronic oral	34.3 mg/kg bw/d	—	—
Beneficial arthropods	honey bees (acute oral toxicity)	clothianidin	—	0.0036 µg/bee	high
		TMG	≥ 152 µg/bee	> 152 µg/bee	virtually non-toxic
		MNG	≥ 153 µg/bee	> 153 µg/bee	virtually non-toxic
		TZMU	≥ 113 µg/bee	> 113 µg/bee	virtually non-toxic
		TZNG	0.89 µg/bee	3.95 µg/bee	moderately toxic

Property	Test substance	Value	Comments
	TZMU	adsorption $K_d = 0.13–0.65$ adsorption $K_{oc} = 46–96$	very high to high mobility
	TMG	adsorption $K_d = 2.4–39$ adsorption $K_{oc} = 525–6159$	low mobility—immobile
Soil leaching	not required—addressed by the adsorption/desorption study		
Volatilization	not required—not volatile		
Field studies			
Field dissipation (relevant Canadian studies)	clothianidin	DT$_{50}$ = 385–1386 d DT$_{90}$ = 1279–4606 d % carry-over = > 80% after 4–6 months	persistent under field conditions no major transformation products formed minor transformation products are TZNG, MNG, TZMU and TMG
Field leaching		No residues detected below 0–45 cm soil depth.	low potential for leaching

Table 4 **Estimated environmental concentrations (Level 1) of clothianidin in drinking water sources**

Groundwater (µg a.i./L)	Reservoir (µg a.i./L)		Dugout (µg a.i./L)	
Annual average concentration[a]	Acute[b]	Chronic[c]	Acute[b]	Chronic[c]
37.1	3	0.28	1.7	0.38

[a] Maximum yearly average for a 20-year simulation

[b] 90th percentile of yearly peaks

[c] 90th percentile of yearly averages

Table 5 **The estimated environmental concentrations (Level 2) of clothianidin in drinking water sources**

	Groundwater (µg a.i./L)	
	90th Percentile	50th Percentile
Ontario	23.7103	22.1301
Quebec	16.48417	13.22874

Table 6 Summary of effects of clothianidin on terrestrial organisms

Group	Organism	Study	NOEL/NOEC	$LD_{50}/LC_{50}/EC_{25}$	Degree of toxicity
Birds	bobwhite quail	acute oral	500 mg/kg bw	> 2000 mg/kg bw	practically non-toxic
	Japanese quail	acute oral	12.5 mg/kg bw	423 mg/kg bw	moderately toxic
	bobwhite quail	acute dietary	309 mg/kg diet	5230 mg/kg diet	practically non-toxic
	mallard duck	acute dietary	646 mg/kg diet	> 5040 mg/kg diet	practically non-toxic
	bobwhite quail	reproductive	205 mg/kg diet	—	—
	mallard duck	reproductive	205 mg/kg diet	—	—
Mammals	rat	acute oral	—	> 5000 mg/kg bw	low
	mouse	acute oral	—	389 mg/kg bw	high
	rat	dermal	—	> 2000 mg/kg bw	low
	rat	inhalation	—	> 5.53 mg/L	low
	rat	reproductive	31.2 mg/kg bw/d for parent	—	high
			9.8 mg/kg bw/d for offspring	—	high
	Beagle dog	chronic oral	34.3 mg/kg bw/d	—	—
Beneficial arthropods	honey bees (acute oral toxicity)	clothianidin	—	0.0036 μg/bee	high
		TMG	≥ 152 μg/bee	> 152 μg/bee	virtually non-toxic
		MNG	≥ 153 μg/bee	> 153 μg/bee	virtually non-toxic
		TZMU	≥ 113 μg/bee	> 113 μg/bee	virtually non-toxic
		TZNG	0.89 μg/bee	3.95 μg/bee	moderately toxic

Table 7 Summary of risk assessment for terrestrial organisms

Organism	Effect	NOEC or NOEL	EEC	Margin of safety	Risk	Mitigative measures
Bobwhite quail	dietary (corn)	27.6 mg/kg/d	367.05 mg/kg/d	0.07	high	label statement
Bobwhite quail	dietary (canola)	27.6 mg/kg/d	195.76 mg/kg/d	0.14	moderate	label statement
Japanese quail	acute (corn)	12.5 mg/kg (1.7 seeds)	65 mg/kg (52 seeds)	0.03	high	label statement
Japanese quail	acute (canola)	12.5 mg/kg/d (1325 seeds)	33.4 mg/kg (2087 seeds)	0.63	moderate	label statement
Mallard duck	dietary (corn)	26.9 mg/kg/d	215.2 mg/kg/d	0.12	moderate	label statement
Mallard duck	dietary (canola)	26.9 mg/kg/d	114.8 mg/kg/d	0.23	moderate	label statement
Bobwhite quail	reproductive (corn)	18.3 mg/kg/d	367.05 mg/kg/d	0.05	high	label statement
Bobwhite quail	reproductive (canola)	18.3 mg/kg/d	195.76 mg/kg/d	0.09	high	label statement
Mallard duck	reproductive (corn)	8.5 mg/kg/d	215.25 mg/kg/d	0.04	high	label statement
Mallard duck	reproductive (canola)	8.5 mg/kg/d	114.8 mg/kg/d	0.07	high	label statement
Small mammal (body weight 0.015 kg)	acute (mouse study)	LD_{50} = 389 mg/kg (310 **corn** seeds)	916 seeds	0.34	moderate	label statement
		LD_{50} = 389 mg/kg (24303 **canola** seeds)	36 650 seeds	0.66	moderate	label statement
Medium mammal (body weight 0.035 kg)	acute (mouse study)	LD_{50} = 389 mg/kg (310 **corn** seeds)	785 seeds	0.4	moderate	label statement
		LD_{50} = 389 mg/kg (24303 **canola** seeds)	31 425 seeds	0.77	moderate	label statement

Organism	Effect	NOEC or NOEL	EEC	Margin of safety	Risk	Mitigative measures
Large mammal (body weight 1.0 kg)	acute (mouse study)	LD_{50} = 389 mg/kg (310 **corn** seeds)	431 seeds	0.72	moderate	label statement
		LD_{50} = 389 mg/kg (24303 **canola** seeds)	17 250 seeds	1.4	no	none required
Honey bees	acute oral	LD_{50} = 0.0036 μg/bee	—	—	residues may pose a risk, if exposed	label statement

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Appendix VI Value summary

Approved use claims for Poncho 600

Crop	Pest	Approved rate (g/ha)		PMRA conclusion	Comments
		Product	a.i.		
Canola Rapeseed	Flea beetle	250 or 666 mL/ 100 kg seed	150 or 400 g/ 100 kg seed	Use fully supported	250 mL/100 kg seeds: For use under low to moderate flea beetle pressure. 666 mL/100 kg seeds: For use under high to extreme flea beetle pressure where extended control is required.
Corn (sweet, field and pop)	Corn rootworm (including northern and western)	166.7 mL/ 80 000 seeds	1.25 mg/ kernel	Use fully supported	The application rate recommended for control of corn rootworm also provides control of other listed corn pests. If corn rootworm is not a target pest, use appropriate lower application rates for control of other listed corn pests.
	Corn flea beetle, black cut worm, seed corn maggot, wireworm	33.3–66.6 mL/ 80 000 seeds	0.25–0.5 mg/ kernel		
	White grub (larvae of European chafer, May/June beetle and Japanese beetle)	33.3 mL/ 80 000 seeds	0.25 mg/kernel		

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